Brain imaging genetics of complex cognitive and neuropsychiatric traits

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IVAN M. CHAVARRIA SILES

BRAIN IMAGING GENETICS OF COMPLEX COGNITIVE AND NEUROPSYCHIATRIC TRAITS

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BRAIN IMAGING GENETICS OF COMPLEX COGNITIVE AND NEUROPSYCHIATRIC TRAITS

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General Introduction

CHAPTER 1

GENERAL INTRODUCTION

During the last three decades the increasing availability of several brain imaging methods that allow studying the morphology and function of the brain have contributed tremendously to the understanding of the cognitive processes, in both healthy subjects and subjects suffering from neuropsychiatric disorders.

Initially, functional imaging methods were expensive and invasive (such as positron and single–photon emission tomography). These methods were rapidly substituted with more cost-efficient non-invasive Magnetic Resonance Imaging (MRI) techniques; currently most brain imaging studies use MRI technology[1]. Thanks to the increased resolution of MRI scanners, we can now obtain whole-brain images with a spatial resolution of about 300-400 μm^2.The fast introduction of higher resolution MRI scanners has been accompanied by a constant improvement of automated statistical methods to quantify and systematically compare morphological and functional differences in brain structures. These methods provide a powerful tool for characterizing individual differences in brain anatomy, as well as in brain activity.

The structural and functional brain measures obtained using MRI are quantitative complex traits that show considerable variation in human populations; both structural and functional measures of the brain have been associated with cognitive function and dysfunction and have provided us with more insight into the underlying neural mechanisms of cognitive traits and disease. The goal of imaging genetics studies is to use brain-imaging data to identify genes for complex behavioral traits; and to characterize the neural systems affected by risk genetic variants to elucidate quantitative and mechanistic aspects of brain function implicated in complex neuropsychiatric disorders[3]. Brain imaging studies can provide information that is not available in classical genetic comparisons of patients and healthy controls, and this approach could eventually be useful to identify potential mechanisms and circuits promoting disease risk[4].

The first imaging genetic studies primarily focused on studying the association between brain morphology and single nucleotide polymorphisms of candidate genes of known function [such as catechol-O-methyltransferase (*COMT*)[5], brain-derived neurotrophic factor (*BDNF*)[6], and disrupted-in-schizophrenia 1 (*DISC1*)[7], among other genes]. Rapidly, the field of imaging genetics moved away from the single candidate gene approach to genome wide association studies (GWAS), just like in other genetic studies. Some GWAS identified a handful of genes associated with psychiatric disorders with unknown functions [for example, the *ZNF804A* gene was associated with schizophrenia[8]], and imaging genetics studies were used to investigate the biological effect of those genes in the brains of subjects with this psychiatric disorder[9-11]. Many of these findings were limited by the very small number of studies reported for each gene, the small sample sizes, the lack of replication, the differences in the methodology of brain morphological measurements, and in the tested anatomical brain regions[3].

The main goal of my PhD thesis is to apply novel statistical methods to analyze brain imaging and genetics data related to cognitive and neuropsychiatric traits to better understand the genetic architecture of these complex traits. The methodological approaches applied in this thesis are designed to increase the power to detect small genetic effects in imaging genetic studies, first by reducing the complexity of the data (i.e. by grouping genes into sets with a plausible biological function, or by grouping the genes in sets of genes previously associated with a specific trait); and second by limiting the imaging phenotypes tested to those that have been previously associated with a specific neuropsychiatric disorder.

To start, I will provide a background of the field of imaging genetics by reviewing the most frequently MRI methods used to study brain structure and function; as well as the most prominent findings in the field of imaging genetics of cognitive neurosciences, and for some neuropsychiatric phenotypes at the time of starting my PhD project (chapter 2). Next, I will present an original research study looking at the effect of a set of genes previously associated with cognitive ability using a novel volumetric brain analysis for genetic studies (chapter 3). The aim in this study is to look at the combined effect that multiple functionally related genes have on the brain structure of healthy subjects. In this study I implemented a novel association analysis model exploring the combined effect of hundreds of genetic variants with small effect

size on gray matter volume across the whole brain; this is a novel approach in the imaging genetic field as most VBMs studies had only looked at the effect of one single or a few genetic variants at the time.

In order to test the potential of imaging genetic studies to identify mechanisms and circuits promoting disease risk, I will present three original research studies on the imaging genetics of schizophrenia. I chose to study this psychiatric disorder because as a psychiatrist I am very interested in this neurodevelopmental disorder which has an enormous impact at personal, societal, medical and economic levels[12], and that affects approximately as many as 1% of the population worldwide[13]. The general aim of these three imaging genetics studies of schizophrenia is to identify plausible genetic links between this complex neuropsychiatric disorder and brain imaging phenotypes.

In two of these studies (chapters 4, and 5) I will use white matter integrity as the phenotype of interest because it provides a good plausible biological endophenotype to explain genetic differences in the risk for schizophrenia[14,15]. The first study will look at the effect that a biologically plausible set of genes (myelination genes) can have on white matter integrity in subjects with schizophrenia and healthy controls. The second study will look at the shared genetics between schizophrenia polygenic risk and white integrity in healthy controls and subjects with schizophrenia.

In the third study, I will use a different approach to study the genetics of schizophrenia, by looking at the effect that sets of genes previously implicated in schizophrenia might have on the total volume of the brain, and on the volume of subcortical brain structures in healthy subjects (chapter 6).

It is important to note that the advances in the imaging genetics field have required collaborations among international consortia, which is needed to reach adequate sample sizes in both the genetics and the imaging fields[4]. The studies on schizophrenia that I will present in this thesis are not an exception, as this work was made possible in part thanks to the collaboration with The MIND Clinical Imaging Consortium (MCIC)[16]; and by using publicly available resources from The Enhancing Neuro Imaging Genetics through Meta-Analysis consortium (ENIGMA), and The Psychiatric Genomics Consortium (PGC).

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Chapter 2

Background: Brain Imaging and Genetics of Cognitive and Neuropsychiatric Traits

CHAPTER 2

BACKGROUND:

BRAIN IMAGING AND GENETICS OF COGNITIVE AND NEUROPSYCHIATRIC TRAITS[1]

The study of how genes can affect brain development and cognition has helped us to better understand the underlying biological mechanisms of normal cognitive traits, and neuropsychiatric disorders.

Genes associated with brain structure and function are of importance for both normal and abnormal cognitive functioning; and vice versa, genes associated with cognitive function, and with neuropsychiatric disorders are also of importance for the development and function of the brain.

In this chapter I will review the most commonly used magnetic resonance imaging techniques to study brain anatomy, connectivity and functionality. I will review how neuroimaging techniques have been used to elucidate the development of the brain across the lifespan and its relation to cognitive function. Also, I'll review the genetic contributions to the field of brain imaging and cognition.

Finally, I will review some of the most consistent findings on the genetics of neuroimaging measures and the effect genetic variation can have on the brain in relation to cognition, and in some neuropsychiatric disorders such as Schizophrenia, Autism, Attention Deficit Hyperactive Disorder, and Alzheimer's Disease.

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1. A modified version of this chapter has been published elsewhere as: Ivan Chavarria-Siles, Guillen Fernandez, and Danielle Posthuma. Chapter 8: Brain Imaging and Cognition. Behavioral Genetics of Cognition Across the Lifespan. Finkel and Reynolds Editors. Springer, **2014**: 235-256. ISBN 978-1-4614-7446-3.

2.1 MRI-based methods to study brain morphology and function

A. Structural MRI

In recent years, a number of unbiased, objective techniques have been developed to characterize neuroanatomical differences *in vivo* using structural Magnetic Resonance Imaging. These techniques can be broadly classified into those that deal with macroscopic differences in brain shape and those that examine the local composition of brain tissue after macroscopic differences have been taken into account (Mechelli et al, 2005). The most commonly used MRI measures to study brain morphology in relation with cognition are: *voxel-base volumetry*, *grey matter cortical thickness and surface,* and measures of *white matter integrity*.

Voxel based brain measures

Voxel-based morphometry (VBM) is one of the most commonly used methods to identify differences in the local composition of brain tissue. This is achieved by spatially *normalizing* all the obtained structural images to a unique stereotactic space; then *segmenting* the normalized images into grey and white matter; followed by *smoothing* the grey and white matter images; and finally performing a statistical analysis to localize significant differences between two or more experimental groups (Ashburner and Friston, 2000). VBM requires several pre-processing steps, as outlined in Box 1.

The VBM analysis output is a statistical parametric map (SPM) showing regions where gray or white matter differs significantly among the experimental groups. These maps can be used to examine differences between e.g. high and low cognitive performers, case and controls for a disease state, or between different genotypic groups.

VBM has also shown to be useful in characterizing subtle changes in brain structure in a variety of diseases associated with neurological and psychiatric dysfunction (Mechelli et al, 2005).

BOX 1

Preprocessing steps of brain images for VBM analyses:

Spatial Normalization: Spatial normalization involves registering the individual MRI images to the same template image. An ideal template consists of the average of a large number of MR images that have been registered in the same stereotactic space.

Segmentation: The spatially normalized images are then segmented into grey matter, white matter, cerebrospinal fluid and three non-brain partitions. This is generally achieved by combining a priori probability maps or "Bayesian priors", which encode the known spatial distribution of different tissues in normal subjects, with a mixture model cluster analysis which identifies voxel intensity distributions of particular tissue types.

Smoothing: The segmented grey and white matter images are smoothed by convolving with an isotropic Gaussian kernel. The size of the smoothing kernel should be comparable to the size of the expected regional differences between the groups of brains.

Cortical thickness and Cortical Surface measures

The human brain grey matter volume is defined as the amount of grey matter that lies between the grey-white interface and the pia mater. The total grey matter volume of the brain is a function of the cortical surface area and cortical thickness; both measurements are globally and regionally independent.

Studies of inter-individual variation in adult brain size have found that those differences in cortical gray matter volume are driven almost exclusively by differences in the cortical surface area rather than cortical thickness, such evidence suggests that surface area and thickness are distinct rather than redundant features of cortical structure. In addition, surface area and cortical thickness have been found to be both heritable, but seem to be genetically uncorrelated (Panizzon et al, 2009).

It is also important to mention that cortical thickness varies considerably between different cortical areas; these variations across the cortex may reflect differences in cell types or neuron densities (Kanai and Rees, 2011).

Several methods have been developed to automatically calculate cortical thickness and surface over the whole brain based on MR images. Cortical anatomy, which is structured as a corrugated two-dimensional sheet of tissue, can be well represented by surface models, which facilitate the analysis of relationships between cortical regions and provide superior visualization. Inter-subject and even interspecies registration can be accomplished using surface-based representations, allowing matching of homologies without relying directly on spatial smoothing as in volume-based methods (Winkler et al., 2010).

Cortical thickness and surface area of great interest to both the study of normal cognitive development as well as a wide variety of neurodegenerative and psychiatric disorders. Changes in the gray matter that makes up the cortical sheet are manifested in normal aging, Alzheimer's disease and other dementias, Huntington's disease, corticobasal degeneration, amyotrophic lateral sclerosis, as well as schizophrenia (Fischl and Dale, 2000).

White matter measures

Diffusion tensor imaging (DTI) is a MRI technique that measures the diffusion of water in tissues. This method measures and quantifies a tissue's orientation and structure. DTI measures are thought to represent brain tissue microstructure integrity and are particularly useful for examining organized brain regions (Taylor et al., 2004). DTI has become one of the most popular MRI techniques in brain research. Diffusion tensor imaging enables visualization and characterization of white matter tracks in two and three dimensions. Since the introduction of this methodology in 1994, it has been used to study the white matter architecture and integrity of the brain (Assaf Y and Pasternak O, 2008).

DTI was rapidly accepted by imaging neuroscientists who saw in it a powerful and unique new tool for exploring the structural connectivity of the human brain.

However, DTI is a rather approximate technique, and its results have frequently been given implausible interpretations. More recently, Diffusion-weighted MRI (DW-MRI) -which only measures the dephasing of spins of protons in the presence of a spatially-varying magnetic field-, has been proposed as the only method capable of mapping the fiber architecture of tissue (*e.g.*, nervous tissue, muscle) *in vivo*. As DW-MRI has matured, an increasing number of software packages have been developed that allow such data to be analyzed in a push-button manner and then derive a p-value which can be interpreted according to the hypotheses being tested (Jones DK et al 2012).

In recent years, DW-MRI has been increasingly used to explore the relationship between white matter structure and cognitive function. DW-MRI has been extensively employed to investigate how individual differences in behavior are related to variability in white matter microstructure on a range of different cognitive tasks and also to examine the effect experiential learning might have on brain structural connectivity. Recent findings suggest that diffusion-weighted imaging might even be used to measure functional differences in water diffusion during task performance (Roberts et al, 2011).

B. Functional MRI

Blood oxygenation level dependent (BOLD) functional MRI

Measuring the BOLD signal in humans using functional MRI (fMRI) provides a non-invasive and large-scale view of neural activation while subjects perform simple or even complex cognitive tasks (event-related BOLD). fMRI has a primary advantage over other techniques (such as PET or SPECT) in neuroscience research due to its non-invasiveness, flexibility, and superior temporal as well as spatial resolution (Serences and Saproo, 2011). This approach has been used to study a remarkable diversity of topics, from basic processes of perception and memory, to the complex mechanisms of economic decision-making and moral cognition (Huettel, 2011).

In recent years, the development of high field MRI methods has resulted in a clearer picture of organization of individual human brains. The dramatic improvement in

the quality of in vivo MRI scanning of human brain by increasing the magnetic field to 7T, and by using a much more sensitive design of radiofrequency receiver coil to detect the MR signal has provided an increase of the signal-to-noise ratio by a factor of 10, allowing whole-brain images with a spatial resolution of only 300-400 μm. In order to meet the goal of *in vivo* mapping of brain's functional areas is necessary to perform systematic high-field MRI studies to provide microscopic anatomical concordance between cortical areas and BOLD (Geyer et al., 2011).

Resting state fMRI

Although the majority of researchers performing functional imaging studies continue to examine changes in brain activity associated while performing a task, some researchers in the field have also studied the spontaneous modulations of brain activity in the absence of an explicit task: resting state fMRI (RS-fMRI). The strength of this method is that it is paradigm-free, as it more or less ignores the cognitive state of the subject; however, this feature also makes the data analysis considerably more difficult than in standard event-related BOLD, as there is not a task that can be used to model the activation pattern (Norris, 2006).

The main difference of this method to regular fMRI is that it looks into differences in connectivity between different parts of the brain and not into brain activity of a particular location. RS-fMRI studies have shown that regional fluctuations of spontaneous brain activity, measured in the absence of an explicit task, are highly organized, and correlated across spatially distributed networks in a manner that recapitulates the topography of task-evoked functional co-activation patterns (Fornito and Bullmore, 2010).

The majority of approaches to analysing RS-FMRI data have thus far been spatially model-driven, with strong *a priori* hypotheses regarding the functional connectivity of a small number of brain regions of interest (ROIs) or individual voxel locations of interest. A characteristic set of co-activating functional systems is found consistently across subjects, stages of cognitive development, degrees of consciousness and even (to some extent) across species. Interestingly, altered resting functioning of large-scale networks has been found in correlation with individual differences in behavioural

performance, as well as in disease and under pharmacological manipulation (Cole et al, 2010). Moreover, individual resting state networks have been shown to be heritable, thus the inter-individual differences found in RS-fMRI studies are expected to be genetically driven (Glahn et al., 2010a).

2.2 Imaging Lifespan Changes of the Human Brain

A. Development of the Brain

The human brain has a particularly protracted maturation, with different tissue types, brain structures, and neuronal circuits having distinct developmental trajectories undergoing dynamic changes throughout the lifespan. The maturation of specific functional systems underlies the development of increasingly sophisticated cognitive functions from childhood to adulthood, including working memory, attention, and cognitive control (Giedd and Rapoport, 2010).

Lenroot et al. (2007) reported the largest longitudinal pediatric neuroimaging study of typically developing children and adolescents (829 scans from 387 subjects, ages 3–27 years); they demonstrated increasing white matter volumes and inverted U-shaped trajectories of grey matter volumes with peak sizes occurring at different times in different regions.

Total cerebral volume follows an inverted U-shape trajectory peaking at age 10.5 in girls and 14.5 in boys. In both males and females, the brain is already at 95% of its peak size by age 6. Across these ages, the group average brain size for males is ~10% larger than for females. This 10% difference is consistent with a vast amount of adult neuroimaging and postmortem studies, and often explained as being related to the larger body size of males. However, it has been found in pediatric subjects that the boy's bodies are not larger than girls' until after puberty. It should be noted that differences in brain size between sexes or other groups should not be interpreted as necessarily imparting any sort of functional advantage or disadvantage. In the case of male/female differences, gross structural measures may not reflect sexually dimorphic differences in functionally relevant factors such as neuronal connectivity and receptor density (Giedd and Rapoport, 2010).

The shape of the age by size trajectories may be related to functional characteristics even more than the absolute brain size. Diffusion tensor imaging studies have shown that anisotropy increases and overall diffusion decreases with age (Cascio et al., 2007).
White matter development is a complex process that continues during childhood and adolescence, whether these changes end in adolescence is not clear. Lebel and Beaulieu (2011) examined longitudinal white matter maturation using diffusion tensor imaging in 103 healthy subjects aged 5–32 years (each subject was scanned at least twice), they assessed the development of 10 major white matter tracts; all tracts showed significant nonlinear development trajectories. Significant within-subject changes occurred in the vast majority of children and early adolescents, and these changes were mostly complete by late adolescence for projection and commissural tracts. Additionally, white matter volume increased significantly with age for most tracts, and longitudinal measures also demonstrated post-adolescent volume increases in several association tracts.

How structural changes impact functional brain maturation is less well understood; understanding dynamic reconfiguration of brain networks between childhood and adulthood requires identifying changes in structural and functional connectivity during this period (Uddin et al., 2011).

Using fMRI approaches in the adult brain, several canonical brain networks have been identified. Three of these can be considered core neurocognitive networks because of their critical roles in high-level cognition: (1) a frontoparietal central executive network (CEN) comprising the dorsolateral prefrontal cortex (DLPFC) and posterior parietal cortex (PPC), related to maintenance and manipulation of information and decision making in the context of goal-directed behavior; (2) a default mode network (DMN), including the ventromedial prefrontal cortex (VMPFC) and posterior cingulated cortex (PCC), associated with internally oriented and social cognition; and (3) a salience network (SN) with nodes in the right fronto-insular cortex (rFIC) and anterior cingulated cortex (ACC), involved in attention as well as interoceptive and affective processes (Sridharan et al., 2008).

How these systems reconfigure and mature with development is a critical question for cognitive neuroscience, with implications for neurodevelopmental pathologies

affecting brain connectivity. Using functional and effective connectivity measures applied to fMRI data, Uddin et al. (2011) examined the interactions within and between the SN, CEN, and DMN. They found that functional coupling between key network nodes is stronger in adults than in children, as are causal links emanating from the rFIC. Specifically, the causal influence of the rFIC on nodes of the SN and CEN was significantly greater in adults compared with children. Developmental changes in functional and effective connectivity were related to structural connectivity along these links.

Diffusion tensor imaging tractography revealed increased structural integrity in adults compared with children along both within- and between-network pathways associated with the rFIC. Their results suggest that structural and functional maturation of rFIC pathways is a critical component of the process by which human brain networks mature during development to support complex, flexible cognitive processes in adulthood.

B. Brain Aging

Good et al. (2001) described the first optimized method of VBM to examine the effects of age on grey and white matter and CSF in 465 normal adults (age 17 to 79). They observed accelerated loss of grey matter volume symmetrically in both parietal lobes and anterior cingulated cortex. Additionally, there is accelerated loss of grey matter concentration in the left middle frontal gyrus, left planum temporale and transverse temporal gyri bilaterally. There was relative preservation of grey matter volume symmetrically in the amygdala, hippocampi, entorhinal cortices, and lateral thalami, with relative preservation of grey matter concentration more diffusely in the thalami. The whole brain volume and grey and white matter partitions were larger in males compared with females.

Furthermore, an interaction of sex with age-related global grey matter decline was observed, with a steeper age-related decline in males. There was not significant interaction of sex with age for CSF or white matter change either globally or regionally. More recently, Peelle et al (2012) replicated some of these findings by assessing age-related changes in gray matter volume in a sample of 420 adults evenly

distributed between the ages of 18–77 years. They found age-related gray matter decline in nearly all parts of the brain, with particularly rapid decline in inferior regions of frontal cortex (e.g., insula and left inferior frontal gyrus) and the central sulcus.
Postmortem and volumetric imaging data suggest that brain myelination is a dynamic lifelong process that, in vulnerable late-myelinating regions, peaks in middle age. BArtzokis et al (2012) assessed the adult lifespan trajectory DTI metrics in 171 healthy subjects 14–93 years of age. Their data suggest that the healthy adult brain undergoes continual change driven by development and repair processes devoted to creating and maintaining synchronous function among neural networks on which optimal cognition and behavior depend.

Resting-state fMRI studies have found that age-related changes in interregional functional connectivity exhibited spatially and temporally specific patterns. During brain development from childhood to senescence, functional connections tended to linearly increase in the emotion system and decrease in the sensorimotor system; while quadratic trajectories were observed in functional connections related to higher-order cognitive functions (Wang et al, 2012)

The aging of the human brain is accompanied not only by changes in cortical and white matter structures, but also by functional activity changes and variable degree of cognitive decline. Finkel et al (2005) used twin data from the Swedish Adoption/ Twin Study of Aging (778 individuals tested 4 occasions over a 13-year-period) to construct four factors from 11 cognitive measures: verbal, spatial, memory, and processing speed. They found that for measures of fluid abilities, the explanatory value of processing speed is paramount for both mean cognitive performance and acceleration with age. They concluded that a significant proportion of the genetic influences on cognitive ability arose from genetic factors affecting processing speed. For measures of fluid abilities, it is not the linear age changes but the accelerating age changes in cognition that share genetic variance with processing speed.

Neurocognitive changes in healthy aging have now been reported for almost 2 decades, of these, executive functions have received the most attention. fMRI studies of executive control processes report robust differences in brain activity between older and younger subjects, particularly under conditions of high executive control

demand**.** The most commonly reported age-related pattern of brain activity during executive function tasks (e.g., working memory, inhibition, and task-switching) is increased recruitment of lateral aspects of the prefrontal cortex bilaterally (Turner and Spreng, 2012).

2.3 Imaging Genetics

A. Genetic Contributions to Human Brain Morphology

Twin studies have been key to determining the contribution of genetic, common and unique environmental influences on variation in brain structures (Posthuma et al., 2000). Structural brain measurements are quantitative traits showing considerable variation in human populations; heritability estimates indicate a strong genetic component contributing to these neuroanatomical phenotypes.

Kaymaz and van Os (2009) extensively reviewed the heritability of gross brain structures; they included 24 studies reporting on the heritability of brain structures in healthy subjects. Gross brain structures show higher heritability rates than specific structures. Brain structure volumes have substantial heritability rates ranging from high (70-95%) for total brain volume, cerebral grey and white matter and corpus callosum, to moderate (40-70%) for the hippocampus, the four lobes (frontal, temporal, occipital and parietal lobe), temporal horn volume, brain parenchyma, white matter hyperintensity and planum temporal asymmetry. Structures formed earlier in development show consistently higher heritability rates than brain structures formed later in development: surface structures seem to be mainly influenced by environmental factors.

Winkler et al (2010) analyzed surface-based and voxel-based representations of brain structure using automated methods, and these measurements were analyzed using a variance-components method to identify the heritability of these traits and their genetic correlations. All neuroanatomical traits were significantly influenced by genetic factors. Cortical thickness and surface area measurements were found to be genetically and phenotypically independent. While both thickness and area influenced volume measurements of cortical grey matter, volume was more closely related to surface area than cortical thickness.

The surface area of the cerebral cortex is a highly heritable trait, yet little is known about genetic influences on regional cortical differentiation in humans. Chen et al (2012) created a human brain atlas based solely on genetically informative data using a fuzzy clustering technique with magnetic resonance imaging data from 406 twins from the Vietnam Era Twin Study of Aging (110 monozygotic and 93 dizygotic pairs, age range: 51-59). With this method they described a previously unidentified parcellation system for the human cortex that reflects shared genetic influences on cortical areal expansion. This human brain atlas may provide novel phenotypes that will have greater statistical power for genome-wide genetic association studies in comparison with traditional cortical parcellations. In addition, they found evidence for a hierarchical, modular, and bilaterally symmetric genetic architecture across hemispheres.

B. Genetic Contributions to Human Brain Function

Functional magnetic resonance imaging is a powerful tool for interrogating the mechanisms of the brain's response to different environmental stimuli. Nonetheless, even with a rigidly standardized stimulus or task, the brain's response is highly variable between people (Blokland et al., 2011). It is, however, challenging to assess the nature of interindividual variation in a spatial process, such as a pattern of neural activity in a fMRI study (Park et al., 2012).

As of today, few studies have addressed the heritability of task-related brain activation. Blokland et al. (2011) reported a voxel-by-voxel genetic model fitting in a large sample of identical and fraternal twins who performed an *n*-back working memory task during fMRI. Patterns of task-related brain response (BOLD signal difference of 2-back minus 0-back) showed moderate heritability, with the highest estimates (40–65%) in the inferior, middle, and superior frontal gyri, left supplementary motor area, precentral and postcentral gyri, middle cingulate cortex, superior medial gyrus, angular gyrus, superior parietal lobule, including precuneus, and superior occipital gyri. Furthermore, high test-retest reliability for a subsample of 40 twins indicated that nongenetic variance in the fMRI brain response is largely due to unique environmental influences rather than measurement error.

Karlsgodt et al. (2010) assessed the genetic contributions to both working memory performance and structural neuroimaging measures focused on the network of these brain regions associated with working memory. Imaging measures included diffusion tensor imaging indices in major white matter tracts thought to be associated with working memory and structural magnetic resonance imaging measures of frontal and parietal grey matter density. Their analyses directly addressed whether working memory performance and neural structural integrity were influenced by common genetic factors. While all cognitive measures, grey matter regions, and white matter tracts assessed were heritable, only performance on a spatial delayed response task and integrity of the superior longitudinal fasciculus (a primary fronto-parietal connection) shared genetic factors.

The default-mode network is diminished during effortful cognitive tasks and it increases when one's mind wonders. This connectivity pattern may be intrinsic to the primate brain, because it is present in sleeping infants and anesthetized nonhuman primates. Aberrant default-mode connectivity has been reported in individuals with neurological and psychiatric illnesses, suggesting that this intrinsic network is sensitive to pathophysiologic alterations in brain function and structure. Although the exact neurophysiologic mechanisms that regulate default-mode connectivity are unclear and likely differ between illnesses, there is growing evidence that genetic factors play a role (Glahn et al., 2010b).

Establishing the heritability of default-mode functional connectivity would authorize the use of resting-state networks as intermediate phenotypes. Glahn et al. (2010b) estimated the importance of genetic effects on the default-mode network by examining covariation patterns in functional connectivity. The heritability for the default-mode functional connectivity was 42%. Although, neuroanatomical variation in this network was also heritable, the genetic factors that influence default-mode functional connectivity and grey-matter density seem to be distinct, suggesting that unique genes influence the structure and function of the network. In contrast, significant genetic correlations between regions within the network provide evidence that the same genetic factors contribute to variation in functional connectivity throughout the default mode.

2.4 Brain Imaging of Cognition

A. Cognition and the Brain

Individual differences in intelligence are strongly associated with many important life outcomes, including educational and occupational attainments, income and health (Batty et al, 2007). The relation between intelligence (measured as Intelligence Quotient [IQ]) and the brain has been studied since the end of the 19th century (Galton, 1888). Structural neuroimaging studies generally report a modest correlation ($r \sim 0.3$) between psychometric measures of intelligence and total brain volume (McDaniel, M., 2005).

The quantity of frontal gray matter is similar in individuals who are genetically alike; intriguingly, these individual differences in brain structure are tightly linked with individual differences in IQ. The resulting genetic brain maps reveal a strong relationship between genes, brain structure and behavior, suggesting that highly heritable aspects of brain structure may be fundamental in determining individual differences in cognition (Thompson, 2001). Jung and Haier (2007) reviewed 37 neuroimaging studies that focused on the relation between intelligence and neuronal networks. They reported a striking consensus of neuroanatomical and functional data suggesting that variations in a certain distributed network predict individual differences in intelligence and reasoning tasks. They described this network as the Parieto-Frontal Integration Theory (P-FIT); the P-FIT model includes the dorsolateral prefrontal cortex (Brodmann's Areas [BAs] 6,9,10,45,46,47), the inferior (BAs 39, 40) and superior (BA 7) parietal lobule, the anterior cingulated (BA 32) and regions within the temporal (BA 21, 37) and occipital (BAs 18, 19). Colom et al. (2009) tested the P-FIT theory in a sample of 100 young healthy adults. Their findings are consistent with the P-FIT theory, supporting the view that general intelligence involves multiple cortical areas throughout the brain.

Links between intelligence and specific regions of the brain may vary according to developmental stage. In the absence of neurological insult or degenerative conditions, IQ is usually expected to be stable across lifespan, as evidenced by the fact that IQ measurements made at different points in an individual's life tend to correlate well (McCall, 1977). Using a longitudinal design, Shaw et al. (2006) found a marked

developmental shift from a predominantly negative correlation between intelligence and cortical thickness in early childhood to a positive correlation in late childhood and beyond, suggesting that the neuroanatomical expression of intelligence in children is dynamic.

More recently, Ramsden et al (2011) tested whether variation in a teenager's IQ over time correlated with changes in brain structure; they used longitudinal assessments of 33 healthy and neurologically normal adolescents first tested when they were 12–16 yr old (mean, 14.1 yr) and then retested the same individuals at age 15–20 (mean, 17.7 yr); in this way they obviated the many sources of variation in brain structure that confound cross-sectional studies. They found that verbal IQ changed with grey matter in a region that was activated by speech, whereas non-verbal IQ changed with grey matter in a region that was activated by finger movements. Surprisingly, their results also suggest the possibility that an individual's intellectual capacity relative to their peers can decrease or increase in the teenage years.

White matter integrity has also been associated with differences in IQ. Chiang et al (2011) reported the first map to demonstrate influences of age, sex, socioeconomic status (SES) and IQ on the heritability of brain fiber architecture. They found moderate but significant modulatory effects of age, sex, intellectual performance (measured by Fluid IQ [FIQ]) and SES, on the heritability of white matter integrity measured by FA. Higher white matter heritability was associated with younger age (adolescents), male sex, higher FIQ, and higher socioeconomic status. They also found that in people with above-average IQ, genetic factors explained over 80% of the observed FA variability in the thalamus, genu, posterior internal capsule, and superior corona radiata. In those with below-average IQ, however, only around 40% FA variability in the same regions was attributable to genetic factors.

The use of fMRI to study cognitive abilities has proven more complex than expected; many functional neuroimaging studies have found that a single brain region can be involved in a broad range of tasks. Therefore, it is unlikely that there is always one core region that is crucial for a particular cognitive function. Instead, a region with a structure that correlates with a behavioral measure needs to be interpreted in the context of the known functions of the region and its role in other, related behavioral tasks (Kanai and Rees, 2011).

B. Imaging Genetics of Cognition

Intelligence is known to be highly heritable, with estimates ranging from 30-40% in childhood and up to 80% in late adulthood (Posthuma et al., 2009). A handful of candidate genes have been associated at least once with cognitive ability, each explaining only about 1-2% of the variance (Deary et al., 2010).

A recent genome-wide association studies for intelligence concluded that intelligence is highly polygenic and thus that many genes of small effects underlie the additive genetic influences on intelligence (Davies G et al., 2011).

The chances of finding these genes may be increased by applying a so-called endophenotype approach[2]. For a measure to be considered an endophenotype, it must be shown to (1) be highly heritable, (2) be associated with the trait, (3) be independent of clinical state, and (4) the measure must co-segregate with the trait within a family (Glahn, 2007).

As a positive correlation between brain size and intelligence has been reported many times, Posthuma et al. (2002) tested whether this correlation was due to shared genes or shared environmental factors. They found high heritability for total brain gray-matter volume, and a correlation between gray-matter volume and intelligence (0.25; $p < 0.05$). They also found a significant correlation between white-matter volume and intelligence (0.24; $p < 0.05$). They concluded that intelligence is related to the volumes of both gray and white matter. Using a twin approach, they decomposed the correlation between brain volumes and intelligence into genetic and environmental components; they showed that the correlation between gray-matter volume and intelligence was due completely to genetic factors and not to environmental factors. The same result was obtained for the correlation between white-matter volume and intelligence.

2. It should be noted that the endophenotype approach relies on the assumption that the genetic basis of endophenotypes is easier to analyze than the categorical classification of an end-phenotype, such as a neuropsychiatric disorder. However, a systematic metanalysis of genetic association studies of endophenotypes showed that while endophenotypes measures may afford greater reliability, it should not be assumed that they will also demonstrate simpler genetic architecture (Flint and Munafo, 2007). The added value of the endophenotype approach thus remains to be proven.

In a subsequent study, Hulshoff Pol et al. (2006) explored the genetic influence on focal GM and WM densities in magnetic resonance brain images of 54 monozygotic and 58 dizygotic twin pairs and 34 of their siblings. For genetic analyses, they used voxel-based morphometry data to explore the common genetic origin of focal GM and WM areas with intelligence. They found that intelligence shared a common genetic origin with superior occipitofrontal, callosal, and left optical radiation white matter and frontal, occipital, and parahippocampal grey matter (phenotypic correlations up to 0.35). The authors suggested that these findings point to a neural network that shares a common genetic origin with human intelligence.

Joshi et al. (2011) analyzed Brain MRI data from 72 young adult twins of age 21–27 yrs (194 dizygotic and 178 monozygotic twins) to identify cortical regions in which grey matter thickness and volume are influenced by genes. They found a strong genetic influence on frontal and parietal regions. In addition, they correlated cortical thickness with full-scale intelligence quotient (IQ), and several regions where cortical structure was correlated with IQ were under strong genetic control. Genetic variants for brain structures and intelligence thus seem to be largely shared. Overall, these findings suggest that genes important for brain structure might also be of importance for intelligence, and vice versa, genes important for intelligence may also be of importance for brain structures.

Under this assumption, Ruano et al. (2010) used an innovative functional gene group analysis to identify if synaptic genes were associated with intelligence; they found that a set of functionally related genes coding for G-proteins are associated with intelligence.

In order to test if the G-proteins group that was found to be associated with intelligence would also explain differences in brain structure, in chapter 3 I will present a study testing the effect of this set of genes on local cerebral grey matter volume using VBM.

2.5 Structural and Functional Brain Imaging of Neuropsychiatric Disorders

The underlying neurobiological pathways of individual differences in human cognitive ability are still poorly understood. Identifying neurobiological pathways for variation in the range of normal cognitive ability could provide important clues to underlying mechanisms of milder but more prevalent forms of altered cognitive functioning. Some of these more prevalent milder cognitive dysfunctions are found in several neurodevelopmental psychiatric disorders such as autism (Mayes and Calhoun, 2008), schizophrenia (Ehrlich et al., 2011), bipolar disorder (Glahn et al., 2010a) and attention deficit hyperactivity disorder (Willcutt et al., 2005). As the life expectancy in the population increases, so does the prevalence of cognitive decline and dementia; up to fifty percent of adults over 85 years of age are currently suffering from cognitive impairtment in the form of Azheimer disease (Hebert et al, 2003).

Neuroimaging endophenotypes are quantitative indicators of brain structure or function that index genetic liability for an illness. These indices will significantly improve gene discovery and help us to understand the functional consequences of specific genes at the level of systems neuroscience (Glahn et al., 2007). Next, I provide a non-exhaustive review of the neuroimaging findings for the most common neuropsychiatric disorders that are accompanied by cognitive dysfunction.

A. Schizophrenia

Schizophrenia is a neurodevelopmental disorder that affects 1% of the population worldwide, and is characterized by hallucinations, delusions, and disorganized thinking and speech. Motivation, cognition, memory, executive functioning, affect, and social communication are all altered in schizophrenia. Before the use of CT and MRI scans, brain abnormalities were based on crude measurements of the post-mortem brains; the major finding of these studies showed enlarged ventricles in patients with schizophrenia.

A large proportion of MRI studies of schizophrenia (80%) also found ventricular enlargement in schizophrenia. Enlargement of the ventricles however is not exclusive of schizophrenia, as this is also observed in hydrocephalus, Alzheimer's disease, and other neurodegenerative disorders where CSF replaces brain tissue. Shenton et al

(2010) reviewed several structural MRI studies of schizophrenia; they found a striking consistency of results showing grey matter abnormalities in chronic schizophrenia including brain regions with the prefrontal, temporal, parietal and occipital lobe. The list of brain regions reported as abnormal is, in fact, quite long and includes nearly all known brain structures.

Despite their impact on imaging phenotypes, the usefulness of candidate genes for understanding schizophrenia is debated because these a-priori hypothesized variants often show an inconsistent effect on the categorical disease phenotype itself. Genome-wide association studies (GWAS) offer an alternative, hypothesis-free way to identify genetic variants associated with the disease; any genetic variant that survives the threshold for genome-wide significance certainly merits study using intermediate imaging phenotypes (Meyer-Lindenberg, 2010).There has been a rapid growth of fMRI studies in schizophrenia, and abnormal activity has been reported in motor tasks, working memory, attention, word fluency, emotion processing, and decision-making. An essential goal of such studies is to demonstrate how failure to activate a neural system leads to behavioral deficits in patients with schizophrenia (Gur and Gur, 2010).

Research on brain activity in schizophrenia has shown that changes in the function of any single region cannot explain the range of cognitive and affective impairments in this illness. Resting state fMRI connectivity measures has been used to predict clinical symptoms and cognitive function. Individuals with schizophrenia showed reduced distal and somewhat enhanced local connectivity between the cognitive control networks. Additionally, greater connectivity between the frontal-parietal and cerebellar regions was robustly predictive of better cognitive performance across groups and predictive of fewer disorganization symptoms among patients. These results are consistent with the hypothesis that impairments of executive function and cognitive control result from disruption in the coordination of activity across brain networks and additionally suggest that these might reflect impairments in normal pattern of brain connectivity development (Repovs et al., 2011).

In chapters 4,5, and 6 of this thesis I will present 3 different imaging genetic studies looking at the effect of genes previously associated with schizophrenia in different brain structures using novel statistical approaches.

B. Autism

Autism spectrum disorder (ASD) is a heterogeneous disorder characterized by abnormal behavior in the spheres of communication, social relatedness, and stereotyped repetitive behaviors within the first three years of life. There are several studies using structural and functional MRI trying to identify brain abnormalities in children with ASD. These studies indicate anatomic differences that although not diagnostic are beginning to elucidate the timing and nature of deviations from typical development (Giedd and Rapoport, 2010).

There are five main findings that can be drawn from the literature on structural MRI of ASD (Chen et al., 2010); 1) volumetric studies reveal that young children with ASD have abnormally increased total brain volume. In addition, juveniles and adults with ASD have reduced corpus callosum volume, and children with ASD have increased amygdala volume. 2) VBM studies consistently report increased grey matter volume in the frontal and temporal lobes in ASD. 3) Cortical thickness studies suggest an increased cortical thickness in the parietal lobes in ASD. 4) Longitudinal MRI studies of ASD suggest abnormal growth trajectories in the frontal and temporal lobes. 5) DTI studies of ASD consistently report corpus callosum abnormalities across a wide age range. Differences in prefrontal white matter, cingulated gyrus, and internal capsule have also been consistently reported.

Apart from structural studies, functional MRI has also been used to understand the neurobiological basis of ASD. Initial studies focused on linear brain-behavior relationships, whereas more recent fMRI studies in ASD have shifted focus towards functional connectivity disturbances. Minshew and Keller (2010) reviewed several fMRI studies of ASD; they consistently found alterations in event-related connectivity in ASD: 1) direct evidence of enhanced activation and connectivity of posterior areas and enhanced reliance on visouspatial abilities for verbal and visual reasoning and reduced frontal systems connectivity. 2) Across studies, it was not uncommon for the cortical location of areas to be shifted slightly, perhaps reflecting recruitment of adjacent cortical areas and lack of the usual cortical specialization for task performance. 3) Resting state connectivity and the default mode network also suggested abnormalities in intrinsic mechanism of thinking, feeling, and behaving, and for the regulation of these processes.

C. Attention-Deficit/Hyperactive Disorder (ADHD)

ADHD is the most common neurodevelopmental disorder of childhood, affecting between 5% and 10% of school-age children and 4.4% of adults. Cross-sectional anatomical imaging studies of ADHD consistently point to the involvement of frontal lobes, parietal lobes, basal ganglia, corpus callosum and cerebellum (Giedd and Rapoport, 2010).

In a meta-analysis of structural MRI findings for ADHD Valera et al (2007), showed that the brain regions most frequently assessed and showing the largest and most significant volume reduction in ADHD patients compared to control subjects include cerebellar areas, in particular the posterior inferior vermis, as well as the splenium of the corpus callosum, total and right cerebral volume and right caudate.
Functional MRI studies have reported abnormal activation in prefrontal cortices (including inferior and dorsolateral regions and cingulated gyrus) and striatum (including caudate and ventral stratium) in individuals with ADHD compared with control subjects (Tomasi and Volkow, 2011). Some of these changes are normalized by stimulant medications such as methylphenidate and amphetamine, supporting the involvement of Dopamine neurotransmission in these functional changes (Rubia et al., 2007).

Most imaging genetic studies of ADHD have focused on dopamine related candidate genes; from 14 imaging genetics studies of ADHD, nine focused on the DAT1 gene and five on the DRD4 gene. The combined findings from these studies could explain how these genes may impact the brain at the structural, functional and biochemical level; however the effect of neither gene is fully understood yet (Durston, 2010).

Several groups have used DTI techniques to study white matter integrity in ADHD; fractional anisotropy has been shown to be significantly reduced in right frontostriatal projections and in the right longitudinal fasciculus, among several other areas of cerebral and cerebellar white matter (Liston et al., 2011).
Resting state functional connectivity studies have reported abnormal signal fluctuations in inferior frontal and superior parietal cortices, cingulate cortex, and cerebellum. Higher resting-state connectivity has been observed in anterior cingulum, pons, insula cerebellum, and thalamus; lower resting-state connectivity was observed

between putamen and posterior parietal cortex and between superior parietal cortices and cingulum (Tomasi and Volkow, 2011).

Finally, there is considerable epidemiological and neuropsychological evidence that ADHD is best considered dimensionally, lying at the extreme of a continuous distribution of symptoms and underlying cognitive impairments. Under this consideration, Giedd and Rapoport (2010) tested whether cortical brain development in typically developing children with symptoms of hyperactivity and impulsivity resembles those found in the ADHD. They found that a slower rate of cortical thinning during late childhood and adolescence, which they previously found in ADHD, was also linked to the severity of symptoms of hyperactivity and impulsivity in typically developing children; this finding suggests neurobiological evidence for the dimensionality of the disorder.

In sum, MRI research in ADHD is a fast developing and very complex field. Every study appears to show differences in brain morphology and in patterns of brain activation between cases and controls; but as of today, the interpretation of such differences is not as straightforward as it may seem.

D. Alzheimer's Disease

Alzheimer's Disease (AD) is the most common cause of dementia in elderly people; Dementia is a disease-related loss of memory and other cognitive abilities of sufficient severity to interfere with activities of daily living (Alzheimer's Association, 2011). AD is a complex disease characterized by an accumulation of β-amyloid (Aβ) plaques and neurofibrillary tangles composed of tau amyloid fibrils associated with synapse loss and neurodegeneration (Weiner et al., 2012). AD is not a normal part of aging; however, old age is its single greatest risk factor (Jack, 2012).

As of today, one of the best-established measurements for the detection and tracking of AD is structural MRI measurements of regional and whole brain tissue shrinkage. Patients have significantly reduced hippocampal and entorhinal cortex volumes, gray matter, and cortical thickness, increased ventricular and sulcal volumes, reduced gray matter or cortical thickness in other cerebral regions, like the precuneus and posterior cingulate, parietal, and temporal cortex (Reiman and Jagust, 2012).

Meda et al (2012) recently summarized the most significant findings on the genetics of AD; the last several decades of research have yielded only 1 genetic risk factor of large effect for late-onset AD: the apolipoprotein-E, with 2 copies of the ε4 allele conferring approximately 6- to 30-fold risk for the disease. More recent genome-wide association studies (GWAS) have identified and replicated 9 additional AD susceptibility genes, including *BIN1*, *CLU*, *ABCA7*, *CR1*, *PICALM*, *MS4A6A*, *CD33*, *MS4A4E*, and *CD2AP*. However, all of these have low effect sizes (odds ratios of 0.87–1.23) and cumulatively account for approximately 35% of population-attributable risk. In order to study alternative methods to understand the imaging genetics of AD, Meda et al (2012) used quantitative intermediate phenotypes derived from magnetic resonance imaging data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database to test for association with gene-gene interactions within 212 known biological pathways. They tested approximately 151 million SNP-SNP interactions for association with 12-month regional atrophy rates using linear regression, with sex, *APOE* ε4 carrier status, age, education, and clinical status as covariates. They found that 109 SNP-SNP interactions were associated with right hippocampus atrophy, and 125 were associated with right entorhinal cortex atrophy; the SNP-SNP interactions that were overrepresented in those interactions are in the calcium signaling, axon guidance, and the ErbB signaling pathway.

2.6 SUMMARY

Magnetic resonance imaging of the brain has allowed us to study the morphology and function of the brain in a non-invasive way. The rapid introduction of high-resolution MRI scanners has been accompanied by a constant improvement of automated statistical methods to quantify and systematically compare morphological and functional differences of diverse brain structures. These methods provide a powerful tool for characterizing individual differences in brain anatomy, connectivity and functionality. Both structural and functional brain measures have been associated with cognitive, affective, and behavioral measures. The field of genetics has started to look at the effect that genetic variants may have on brain structure and function; studying how genes can affect brain development and cognition has helped us to better understand the underlying biological mechanisms of cognitive traits and neuropsychiatric disorders.

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Chapter 3

Brain Imaging Genetics of Genes Associated with Brain Development and Cognitive Ability

CHAPTER 3

BRAIN IMAGING GENETICS OF GENES ASSOCIATED WITH BRAIN DEVELOPMENT AND COGNITIVE ABILITY[3]

3.1 ABSTRACT

The heterotrimeric G proteins have been well established as key regulators of neuronal growth, differentiation and function. More recently, the heterotrimeric G-protein genes group was associated with general cognitive ability. G-protein-coupled signal transduction mediates most cellular responses to hormones and neurotransmitters; this signaling system transduces a large variety of extracellular stimuli into neurons and is the most widely used mechanism for cell communication at the synaptic level.

Although heterotrimeric G proteins are linked to both cognitive ability and neuron signaling, it is unknown whether heterotrimeric G proteins are also important for brain structure. We tested for association between local cerebral grey matter volume and the heterotrimeric G-protein genes group in 294 subjects; a replication analysis was performed in an independent sample of 238 subjects.

Voxel-based morphometry revealed a strong, replicated association between two genes encoding heterotrimeric G-proteins with specific, local increase in medial frontal cortex volume, an area known to be involved in cognitive control and negative affect. This finding suggests that heterotrimeric G proteins might modulate medial frontal cortex grey matter volume. The differences in grey matter volume due to variations in genes encoding G-proteins may be explained by the role of G-proteins in prenatal and postnatal neocortex development.

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3.2 INTRODUCTION

Heterotrimeric G-proteins (G-proteins) are the molecular switches that turn on intracellular signalling cascades in response to the activation of G-protein-coupled receptors (GPCRs) by extracellular stimuli (hormones and neurotransmitters) (Oldham and Hamm, 2008). The G-proteins are abundantly expressed in the brain and are necessary for normal neuronal growth, cortical development and brain function (Bromberg et al., 2008;Ma'ayan et al., 2009;Moers et al., 2008;Sanno et al., 2010;Tahirovic et al., 2010;Tanaka et al., 2007).

We previously reported a strong, replicated association of the group of genes encoding synaptic G-proteins with cognitive ability, using a functional gene group analysis (Ruano et al., 2010). It is unknown however, how G-proteins may affect cognitive ability.

Studies using magnetic resonance imaging (MRI) have consistently reported associations between brain morphometry and cognitive ability; the majority of these studies used voxel-based morphometry (VBM) to measure local grey and white matter volume across the entire brain. Large inter-individual variation in total and regional brain volume exists, which is mainly due to differences at the genetic level. Twin studies have shown that many aspects of brain structure and function are highly heritable; genetic factors account for most of inter-individual differences, with heritability estimates ranging from 82% for grey matter to 88% for white matter (Baare et al., 2001;Thompson et al., 2001).

Even from early age on, MRI studies on neonates have shown high heritability of local and total brain morphology (Gilmore et al., 2010). Despite the generally high heritability of brain morphology, even the most highly associated common genetic polymorphisms explain less than 1-5% of the variation in most brain measurements (Thompson et al., 2010).

It is well-known that the correlation between local brain volume and cognitive ability (Rushton and Ankney, 2009;Witelson et al., 2006) is largely explained by correlations at a genetic level (Posthuma et al., 2002), genes that are important for cognitive ability may also be important for local brain volume. The group of genes

encoding G-proteins poses an excellent candidate gene group for association with local brain volumes due to their known expression in the brain and their role in cortical development and neuronal growth. We thus set out to test whether genes encoding G-proteins explain individual differences in local brain volume using two relatively large datasets.

3.3 MATERIALS AND METHODS

Subjects

A total of 532 healthy individuals were included from the Brain Imaging Genetics (BIG) study at the Donders Institute for Brain, Cognition and Behavior of the Radboud University Nijmegen Medical Center, The Netherlands. All participants included in the study were right-handed and of European Caucasian descendent. The regional medical ethics committee approved the study and all participants gave written informed consent.

In order to avoid the well-known inter-scanner confounding (Focke et al., 2011) the sample set was divided into two groups according to the MRI scanner used; 294 subjects were scanned in a 3 Tesla (T) Siemens scanner (Erlagen, Germany) and 238 subjects were scanned in a 1.5T Siemens scanner (Erlagen, Germany).

To increase the power to detect association, the larger sample was used as a discovery sample and the smaller sample was used as an independent sample for replication. We only included subjects aged 18 to 36 years old as for this age group the effects of brain development or aging is negligible. The demographics of the two samples are described in supplementary table S1.

Genotyping

DNA was extracted from saliva using the Oragene DNA sample collection kit (DNA Genotek, Kanata, Canada). Genotypes were obtained using an Affymetrix GeneChip SNP 6.0 (AFFY6) array (Santa Clara, California, United States). The call rate threshold was set at 90% for the arrays, with an average call rate of 96.3%.

Single Nucleotide Polymorphisms selection

All known Single Nucleotide Polymorphisms (SNPs) within the heterotrimeric G-protein genes (supplementary table S2) that are expressed in the brain were retrieved in batch from the SNP database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/projects/SNP/) and were subsequently filtered for SNPs that were genotyped on the AFFY6 platform.

The G-protein genes group expressed in the brain is composed of 27 genes (supplementary table S2), of which 25 (including 677 SNPs) were represented on the AFFY6 platform (no SNPs were available on this platform for the *GNB2* and *GNG3* genes). We excluded all genotyped SNPs that showed a minor allele frequency (MAF) less than 5% as well as genotyped SNPs that were in complete linkage disequilibrium (LD) with another SNP within the same gene. MAF and LD were calculated using PLINK-v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/). In total, we included 502 SNPs within 25 genes in the final analysis (supplementary table S2).

Brain imaging

Image acquisition

T1-weighted structural MRI data were acquired at either 3T (n = 294) or 1.5T (n = 238). All 1.5T images were acquired at 1.5T Siemens Sonata and Avanto scanners (Siemens, Erlangen, Germany), using small variations to a standard T1-weighted 3D MPRAGE sequence (TR 2300 ms, TI 1100 ms, TE 3.03 ms, 192 sagittal slices, field of view 256 mm). These variations included a TR/TI/TE/slices of 2730/1000/2.95/176, 2250/850/2.95/176, 2250/850/3.93/176, 2250/850/3.68/176, and the use of GRAPPA parallel imaging with an acceleration factor of 2. All scans covered the entire brain and had a voxel size of 1x1x1 mm^3. All 3T images were acquired at 3T Siemens Trio and TrioTim scanners (Siemens, Erlangen, Germany), using small variations to a standard T1-weighted 3D MPRAGE sequence (TR 2300 ms, TI 1100 ms, TE 3.93 ms, 192 sagittal slices, field of view 256 mm). These variations included TR/TI/TE/slices of 2300/1100/3.03/192, 2300/1100/2.92/192, 2300/1100/2.96/192, 2300/1100/2.99/192, 1940/1100/3.93/176, 1960/1100/4.58/176, and the use of GRAPPA parallel imaging with an acceleration factor of 2.

Conversion

Raw DICOM MR imaging data were converted to NIFTI format using the conversion as implemented in SPM5.

Brain Volume

Normalization, bias-correction, and segmentation into gray matter, white matter and cerebrospinal fluid were performed using the VBM toolbox in SPM using priors (default settings). This method uses an optimized VBM protocol (Ashburner and Friston, 2000;Good et al., 2001) as well as a model based on Hidden Markov Random Fields (HMRF) developed to increase signal-to-noise ratio (Cuadra et al., 2005). Total volume of gray matter, white matter and cerebrospinal fluid were calculated by adding the resulting tissue probabilities. Brain volume was defined as the sum of white matter and gray matter volume.

Voxel-Based Morphometry preprocessing

Diffeomorphic image registration was performed using the DARTEL toolbox in SPM (Ashburner, 2007). First, all images were realigned to templates created from 556 in-house datasets. Second, Jacobian scaled ('modulated') images were calculated and subsequently transformed to MNI space using affine transformation. Finally, all data were smoothed with an 8 mm FWHM Gaussian smoothing kernel.

Statistics

A full-factorial ANCOVA was applied using genotype as factor and participants' age, gender, total brain volume, and scan protocol were added to the model as covariates. Statistics were first done using an F-contrast and corrected for non-stationarity for cluster statistics (Hayasaka et al., 2004) at $p < 0.05$ (FWE corrected for whole brain). For the most significant findings a T-statistic was applied to determine the direction of the effect. For the association analysis we used a mass univariate model for pathway-based approaches to imaging genetics association studies as previously described by Inkster et al. (Inkster et al., 2010).

In summary, for each of the 502 SNPs we performed a separate VBM analysis for grey matter using standard tools in SPM5, This approach was used because the

G-proteins genes are expressed ubiquitously in the whole brain and might show associations with many different brain regions. Two or three level group factor was used for genotype status: (a) for SNPs with a MAF >0.31 (more than 10% of subjects with the minor allele) a 2 degrees of freedom genotypic model was applied using an additive parameterization with a centered covariate; (b) for SNPs with MAF <0.31 (less than 10% of subjects with the minor allele) a recessive model merging the rare homozygous and heterozygous groups was used.

In order to find associations between the G-proteins genes group and variations in grey matter volume, we tested for associations between individual SNPs and grey matter variations. Cluster size of voxels was inferred using the toolbox "NS" implemented on SPM5 (Hayasaka et al., 2004). Results were corrected for non-stationarity and assessed initially at $p<0.001$ uncorrected for the whole brain volume. Subsequently cluster statistics were applied using Family-Wise Error (FWE) correction and results were considered significant at p-(FWE) $<5x10^{-4}$, which is the Bonferroni adjusted p-(FWE)-value for the total number of SNPs tested in the whole analysis (502 SNPs).

In order to replicate the findings of the discovery sample, we performed the same analysis in our replication sample, using only the significant findings from the discovery sample as regions of interest (ROIs) to apply a Small-Volume Correction (SVC) to the results using the MarsBar toolbox implemented in SPM5 (http://marsbar.sourceforge.net/). This allows to specifically test the same brain regions in both samples, selecting only the cluster found to be significant in the discovery sample and testing it for association in the replication sample. Results were considered significant at p-(SVC) <0.007, this is the adjusted p-(SVC)-value for the total number of SNPs tested in the replication analysis (7 SNPs).

In summary, multiple testing corrections were applied at different levels: first, adjusting for testing multiple voxels in the whole brain was implemented using Family-Wise error (FWE) correction; secondly adjusting for testing multiple SNPs using Bonferroni correction, and finally the gold standard of replication analysis was carried out to rule out any remaining false positive associations.

3.4 RESULTS

Using VBM of T1-weighted structural MRI measurements we tested the association between local cerebral grey matter volume variations and 502 SNPs spanning 25 G-proteins genes. We identified seven genes (*GNG2, GNAQ, GNA14, GNA15, GNAO1, GNAL, GNB5*) in which (after Bonferroni correction for all the 502 SNPs tested) at least one SNP was significantly associated with grey volume variations (denoted as *p*-value Group$_{corrected}$ in Table 1).

Four out of these seven genes (*GNG2, GNAQ, GNA15, GNA14*) mapped predominantly to grey matter differences in the same area, namely the medial frontal cortex (Figure 1A); the associated areas in the medial frontal cortex overlapped in Brodmann area 32 for three of these genes: GNG2, GNAQ and GNA15 (Figure 1B). Two genes (*GNAO1* and *GNAL*) mapped to significant differences in grey matter in the temporal lobe (Brodmann areas 20, 21, 28, 34) and one gene (*GNB5*) showed association for grey matter differences in a region in the occipital lobe (Brodmann area 18).

In order to replicate these findings, we tested the SNP that had the lowest structural association p-value in the discovery sample for each of the seven genes that were significantly associated with grey matter volume variation in an independent sample of 238 subjects. We found replicated, regional association of two genes: *GNG2* and *GNAQ* gene with differences in local grey matter volume in the medial frontal cortex (denoted as SVC *p- value* in Table 1). No significant replications were observed for the temporal or the occipital lobe associations found in the discovery sample. If an additional Bonferroni correction is applied for the 7-replication tests, only the SNP rs11851703 on GNG2 gene would survive the α= 0.007 for this additional correction.

Using a T-test analysis (Supplementary Figure 1) of the most significant replicated finding we identified that the rare allele of the SNP rs11851703 (MAF= 0.051) is associated with an increased volume of grey matter in the medial frontal cortex in both the discovery and the replication sample (Figure 1C). For the replication sample, this SNP explains almost 10% of the variance observed in the grey matter volume for the ROI obtained from the discovery analysis (T= 5.10; DF= 237; Cohen's d= 0,6625; Effect-size r= 0,3144; r^2=0,098).

TABLE 1

Association analysis of G-proteins genes with grey matter volume

Discovery sample n= 294										Replication sample n= 238	
SNP*			Cluster					*P values*			
rs number	MAF	Gene [a]	Brain Region (Brodmann Area)	Size	Local Maximum X	Y	Z	FWE $_{corrected}$ [b] $\alpha = 5 \times 10^{-04}$	Group $_{corrected}$ [c] $\alpha = 0.05$	MAF	SVC *P-values*[d] FWE $_{corrected}$
rs11851703	0.0514	GNG2 (40)	Medial Frontal Cortex (24, 32)	1302	-2	30	25	5.05×10^{-05}	0.02534	0.0506	0.002
rs4745679	0.4851	GNAQ (47)	Medial Frontal Cortex (8, 32)	2047	15	9	51	9.27×10^{-07}	0.00046	0.4838	0.034
rs2238628	0.0864	GNA15 (8)	Medial Frontal Cortex (24, 32)	2001	-8	4	34	1.15×10^{-05}	0.00575	0.0860	NS
rs4745639	0.1476	GNA14 (85)	Medial Frontal Cortex (10, 11)	3369	-10	61	3	1.36×10^{-06}	0.00682	0.1579	NS
rs1382362	0.4012	GNAO1 (43)	Temporal lobe (28, 34)	2574	25	0	-21	7.60×10^{-07}	0.00038	0.3801	NS
rs17515178	0.1583	GNAL (48)	Temporal lobe (20, 21)	866	-65	-13	-24	4.25×10^{-05}	0.02132	0.1565	NS
rs12396	0.3835	GNB5 (9)	Occipital lobe (18)	902	31	-85	-15	5.77×10^{-05}	0.02897	0.4013	NS

*Only SNPs with significant associations with brain structural variation are shown, for cases in which multiple SNPs related to the same gene were significantly associated, the SNP that had the lowest structural association *p*-value is shown. MAF= minimum allele frequency.

[a] In parenthesis the number of SNPs tested for that specific gene.

[b] FWE-corrected p-values for the map-wise cluster based association test.

[c] FWE-corrected p-values after applying Bonferroni correction based on 502 SNPs tested in the whole gene group analysis.

[d] SVC-corrected p-values for the association test in the replication sample.

NS= No Suprathreshold Cluster association.

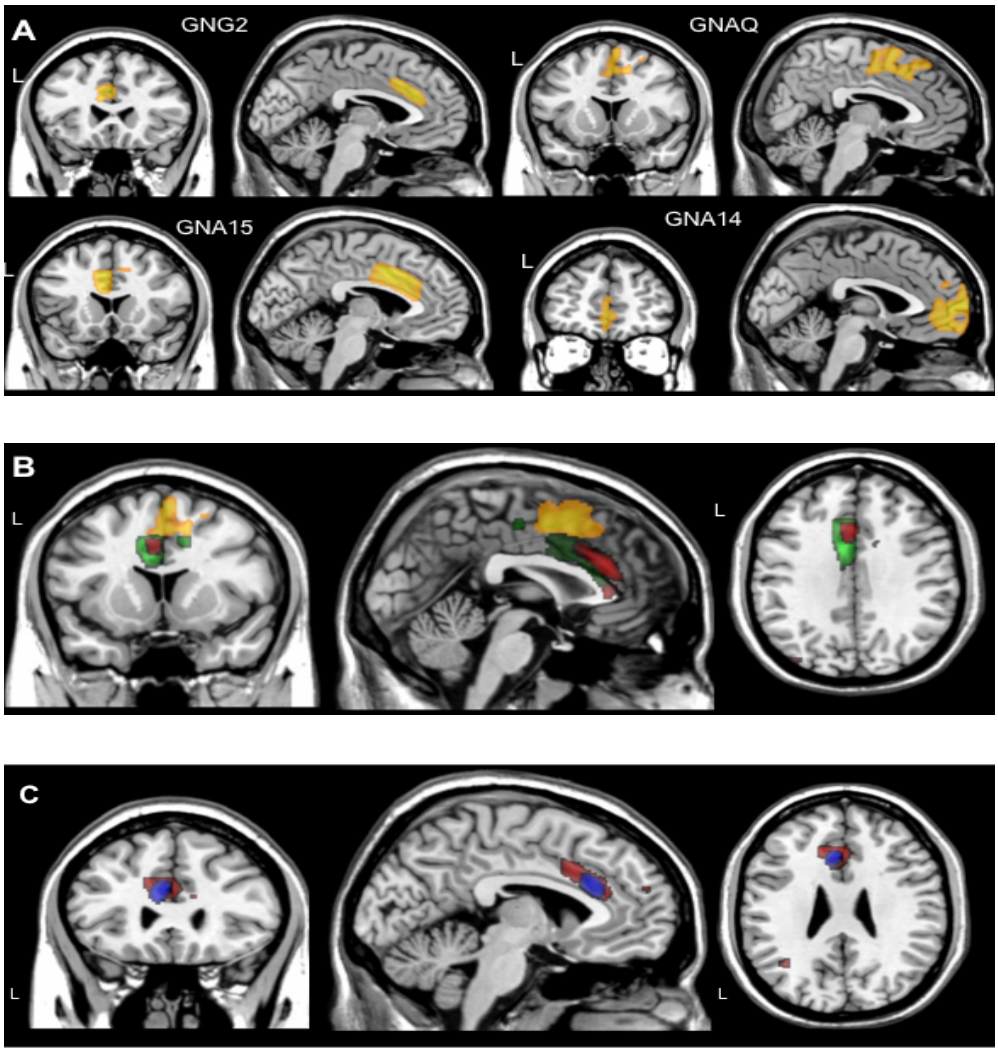

Figure 1. Anatomical locations of SNP-association clusters of G-proteins genes that showed significant FWE corrected P values of local gray matter differences in the medial frontal cortex. (A) The anatomical cluster for each gene corresponds to the SNP that had the lowest significant association P values after Bonferroni correction in the discovery sample: GNG2 (rs1185703), GNAQ (rs4745679), GNA15 (rs2238628), GNA14 (rs4745639). (B) Overlap of 3 clusters of SNP-associated G-proteins genes that showed significant association with local gray matter differences in the medial frontal cortex (Brodmann areas 24 and 32). Red Cluster: GNG2 (rs11851703); yellow cluster: GNAQ (rs4745678); green cluster: GNA15 (rs2238628). (C) Anatomical location of the overlapping cluster of SNP rs11851703 (GNG2 gene) in the discovery sample (red cluster) and the replication sample (blue cluster).
‘‘L’’: left hemisphere.

3.4 DISCUSSION

In this study we investigated whether common genetic variants in the group of genes encoding G-proteins are related to local grey matter volume variations. We found that SNPs in seven G-proteins genes (*GNG2, GNAQ, GNA14, GNA15, GNAO1, GNAL, GNB5*) were significantly associated with differences in three brain regions: the medial frontal cortex, the temporal lobe and the occipital lobe. Interestingly, variants in four of the significantly associated G-proteins genes (*GNG2, GNAQ, GNA15, GNA14*) were associated with grey matter changes in a overlapping region of the medial frontal cortex. We conducted a second study to verify our initial result in an independent sample, and were able to replicate the association for two of the four genes in exactly the same brain region of the medial frontal cortex as the discovery sample.

When interpreting results from an imaging genetics' study, two main issues are important. The first issue is to show how likely a reported significant association is, and to determine whether it might simply be due to chance. Lately, concerns have risen (Meyer-Lindenberg et al., 2008;Silver et al., 2010) about multiple testing issues that could lead to high false positives rates in imaging genetics (in our case a whole brain study of 502 SNPs across 600,000 voxels requires about $3x10^8$ separate tests). Silver et al. (Silver et al., 2010) recently compared different statistical approaches in neuroimaging genetics using voxel-based morphometry data and defined clear guidelines in order to reduce the probability of obtaining false positives results. In our study we followed these guidelines and used a nonparametric non-stationary cluster size inference with a relatively high cluster-forming threshold (α= 0.001) on images smoothed a 8mm Gaussian kernel [instead of the 12mm Gaussian kernel smoothing recommended by Silver et al. (2010); we used a 8mm Gaussian kernel because the smoothing kernel is always a trade-off between spatial accuracy (with as much as 12 mm you cannot claim that some effect is in a small structure) and statistical validity].

However, the likelihood of false positive findings is reduced by the discovery-replication design that we used; with these parameters, rejection rates (at a nominal significance level of 5%) are expected to be well-controlled as suggested by Silver et al. (2010). In addition to the statistical parameters used to determine corrected significance while accounting for spatial dependences between voxels (FWE whole

brain corrected), we only considered a finding to be statistically significant if the nominal *p*-value was still significant after Bonferroni correction for 502 SNPs tested (Table 1), which is known to be overly conservative, especially when tests are non-independent. Even after rigorous multiple testing correction in our discovery sample, associations remained significant for four G-proteins genes with the medial frontal cortex, a brain region that is known to be involved in cognitive control and negative affect (Shackman et al., 2011).

In genetic association studies, replication has become the gold standard to show that significant results, even after multiple corrections, are genuine. Most neuroimaging studies to date have been severely underpowered to detect associations; replicating association results requires even larger samples, rendering the need for phenotyping standardization and data sharing even greater (Congdon et al., 2010). For imaging studies this is not yet common practice, mainly because of time and financial investments. However, more, large scaled MRI datasets are becoming available allowing for independent replication testing. We used both a discovery dataset and an independent replication dataset and showed replicated association with medial frontal cortex volume for two genes encoding G-proteins.

A second issue that is important when interpreting results from an imaging genetics study is to understand how common the discovered genetic association with a brain region is. That is, if a given number of randomly drawn genes (i.e. 100 groups of 500 SNPs) would be tested against the same number of voxels as used here, how likely is it that such genes would also show significant association? Such an approach will require performing 50,000 individual VBM analyses on 600,000 voxels.

If this is likely, it means that besides the original genetic association is, although still bona vida, multiple other effects might be just as important: just one effect amongst very many other contributing effects. If not, it seems justified to conclude that the variation in the associated genes is one of the major genetic factors influencing this trait. Determine whether other genetic effects might be as important as the effect of the G-proteins would fit in an exploratory study. However, our current study was a hypothesis driven study focusing on a functional group of genes with high prior validity.

It is important to notice that the rare allele of SNP rs11851703 has a relatively large effect size (r^2= 0.098) in our replication analysis. This could be explained by the fact that our model was limited to test univariate main effects and it is only adequately powered to detect relatively large effects. Even though, higher-order modeling (multivariate, non-additive) is outside the scope of this paper, the field of imaging genetics should work towards developing reliable statistical methods to allow relating the combine effects of large number of genetic variants on equally multidimensional neuroimaging phenotypes (Meyer-Lindernberg, 2011).

The complexity of the high-volume data sets generated in imaging genetics is prone to produce too many false positives or false negatives. Even when our approach allowed us to prevent high rate of false positive findings, this approach might also increase the risk of type II errors, which at the end might represent the mayor limitation of this study.

The multiple layers of statistical protection and the independent replication analysis strengthen the conviction that the observed associations of grey matter volume variation in the medial frontal cortex with markers in GNG2 and GNAQ genes are genuine. For the most significant finding, in *GNG2*, we found that carriers of the rare allele of SNP rs11851703 in both the discovery and the replication sample had an increased grey matter volume of the medial frontal cortex, specifically in the anterior cingulate cortex (Brodmann areas 24 and 32), compared to non-carriers.

The cingulate cortex has been implicated in both cognitive control and negative affect (Bush et al., 2000;Shackman et al., 2011). The anterior cingulate cortex has also been implicated in cognitive processes such as attention and executive functions, including performance monitoring (Botvinick et al., 2001;Braver et al., 2001).

An increased grey matter volume as we found in the dorsal medial frontal cortex may explain differences in cognitive processes. During normal brain development the maturation of the medial frontal cortex facilitates improvements in performance monitoring, an ability that is necessary to learn from experience, a critical feature of human cognition (Fitzgerald et al., 2010); this maturation could be modulated genetically by genes that regulate brain development and at the end creates differences in grey matter volume in this region.

The differences in grey matter volume due to variation in genes encoding G-proteins may be explained by the role of G-proteins in prenatal and postnatal neocortex development (Bromberg et al., 2008;Ma'ayan et al., 2009;Moers et al., 2008;Sanno et al., 2010;Tahirovic et al., 2010;Tanaka et al., 2007). Multiple G-proteins-coupled receptors regulate the migratory activity of neurons by modulating the activity of Rac and/or Rho (Sah et al., 2000). The activation of RhoA via G-proteins-coupled receptors is primarily mediated by the ubiquitously expressed G-proteins GNA12 and GNA13; these two G-proteins were found to be required for the proper positioning of migrating cortical plate neurons and Purkinje cells during brain development (Moers et al., 2008). The RhoA subfamily within the Ras superfamily of GTP-binding proteins was found to determine neuronal density during postnatal neocortex development by regulating the G-proteins' activity (Sanno et al., 2010).

Several signaling cascades activated by the G-proteins have also been implicated in genetic disorders that show different levels of cognitive ability impairment. Mutations in some of the genes encoding proteins of the signaling cascades activated by the G-proteins have been associated with developmental disorders that feature different levels of cognitive deficits (Cesarini et al., 2009).

In conclusion, our study shows that two members of the G-proteins genes explain individual differences in medial frontal cortex volume, a brain region known to be involved in cognitive control and negative affect. This suggests that G-proteins may also be important for disorders such as schizophrenia or ADHD where cognitive dysfunction is a prominent feature and that G-proteins may be responsible for at least part of the reported genetic association between brain volume and cognitive ability (Posthuma et al., 2002).

3.5 ACKNOWLEDGMENTS

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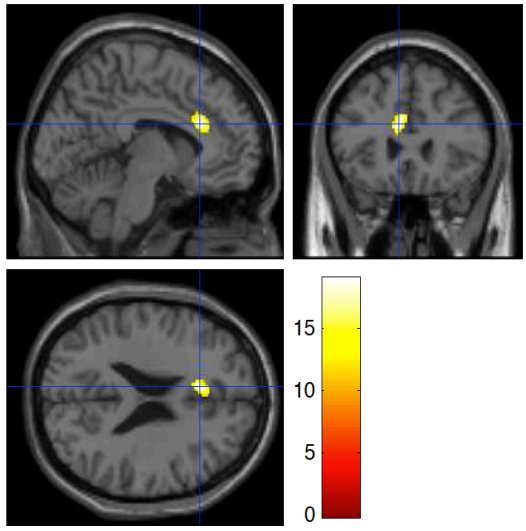
3.7 SUPPLEMENTARY MATERIAL

Supplementary Figure 1.
Replication Analysis SNP rs11851703

A. F Statistics rs11851703 (Replication Sample)

Statistics: *search volume: image mask: ./rs11851703_mall_olume.img,1*

set–level		cluster–level			voxel–level					mm	mm	mm
p	c	$p_{corrected}$	k_E	$p_{uncorrected}$	$p_{FWE-corr}$	$p_{FDR-corr}$	F	$(Z_=)$	$p_{uncorrected}$			
0.0007			139		0.002	0.001	19.04	4.13	0.000	−7	26	23
			217		0.003	0.001	17.81	3.99	0.000	−45	53	3
			33		0.011	0.001	15.09	3.65	0.000	−28	−37	40
			66		0.014	0.001	14.48	3.57	0.000	−40	9	35
			27		0.044	0.001	11.95	3.22	0.001	−30	−16	17
			6		0.046	0.001	11.86	3.21	0.001	35	49	18
			2		0.049	0.001	11.71	3.19	0.001	30	−83	−3



B. T Statistics rs11851703 (Replication Sample)

Statistics: *search volume: image mask: ./rs11851703_mall_olume.img,1*

set–level		cluster–level			voxel–level					mm	mm	mm
p	c	$p_{corrected}$	k_E	$p_{uncorrected}$	$p_{FWE-corr}$	$p_{FDR-corr}$	T	$(Z_=)$	$p_{uncorrected}$			
0.0004		0.003	139	0.052	0.000	0.000	5.10	4.98	0.000	−7	26	23
		0.001	217	0.018	0.000	0.000	4.94	4.82	0.000	−45	53	3
		0.010	66	0.164	0.001	0.000	4.45	4.37	0.000	−40	9	35
		0.041	6	0.690	0.003	0.000	4.03	3.97	0.000	35	49	18

Chapter 4

Myelination Genes and White Matter Integrity in Schizophrenia

CHAPTER 4

MYELINATION GENES AND WHITE MATTER INTEGRITY IN SCHIZOPHRENIA[4]

4.1 ABSTRACT

Disruptions in white matter (WM) tract structures have been implicated consistently in the pathophysiology of schizophrenia. Global WM integrity - as measured by fractional anisotropy (FA), is highly heritable and may provide a good endophenotype for genetic studies of schizophrenia. WM abnormalities in schizophrenia are not localized to one specific brain region, but instead reflect global low-level decreases in FA coupled with focal abnormalities. In this study we sought to investigate whether functional gene-sets associated with schizophrenia are also associated with white matter integrity. We analyzed FA and genetic data from the Mind Research Network Clinical Imaging Consortium to study the effect of multiple oligodendrocyte gene-sets on schizophrenia and white matter integrity using a functional gene set analysis in 77 subjects with schizophrenia and 104 healthy controls.

We found that a gene-set involved in myelination was significantly associated with schizophrenia and FA. This gene set includes 17 genes that are expressed in oligodendrocytes and one neuronal gene (*NRG1*) that is known to regulate myelination. None of the genes within the gene-set were associated with schizophrenia or FA individually, suggesting that no single gene was driving the association of the gene-set. Our findings support the hypothesis that multiple genetic variants in myelination-related genes contribute to the observed correlation between schizophrenia and decreased white matter integrity as measured by FA.

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4.2 INTRODUCTION

Schizophrenia is a chronic disabling disorder that affects approximately 1% of the population worldwide with devastating personal, social, and economic effects. Schizophrenia is highly heritable and multiple genes with small to modest effect size in combination with unknown environmental factors are involved in its pathogenesis[1-4]. Recent reports suggest the involvement of the neuronal calcium signaling pathway[3], as well as the involvement of specific glial functions such as those related to oligodendrocyte function[5]. The latter is of particular interest when placed in the context of brain imaging results for schizophrenia.

Oligodendrocytes are glial cells responsible for the synthesis of the myelin sheath, which supports and isolates neuronal axons forming the white matter in the brain. Disruptions in white matter tract structures have been consistently implicated in the pathophysiology of schizophrenia; a number of diffusion tensor imaging (DTI) studies in schizophrenia have implicated white matter abnormalities in various brain regions[6-10]. Although there is a lack of consistency in the spatial localization of the brain regions showing reduced FA[11], in this regard it has been found that WM abnormalities are not localized to a specific brain region, but instead reflect a diffuse process with widely dispersed focal reductions in FA that vary spatially among individuals[12-14]. Of all DTI parameters examined so far, fractional anisotropy of water diffusion has the highest reported heritability[15].

It's unclear how white matter integrity abnormalities relate to the underlying genetic architecture of Schizophrenia, nevertheless the endophenotypic importance of fractional anisotropy for schizophrenia is further supported by a recent studies showing that many brain regions showing significant decrease in FA in subjects with schizophrenia (including childhood-onset schizophrenia) were also decreased similarly but with smaller effects in their relatives; with a continuous FA decrease from healthy subjects to relatives to subjects with schizophrenia[16-17].

Thus, global white matter integrity (as measured by FA) may provide a good biological endophenotype to explain genetic differences in the risk for schizophrenia [18-19]. In this study we sought to investigate whether oligodendrocyte gene-sets associated with schizophrenia are also associated with white matter integrity.

4.3 METHODS

Gene set definition

When performing a gene set association analysis the most critical step is the definition of the gene sets. For this study we used a modified version of the oligodendrocyte gene-sets derived from expert curated glial gene lists described in detail by Goudriaan et al.[5]. The main goal of that study was to create list of genes that were mainly expressed in glial cells. The authors of that study defined their gene-sets based on an in-depth literature study and selected glial genes based on microarray gene expression patterns. The microarray sources included 1) microarray studies comparing different mouse central nervous system cell-types; 2) microarray studies of whole human brain material; 3) microarray studies of upregulated genes in human; 4) mouse microglia after stimulation of these cells with pro-inflammatory stimuli; 5) and a group of oligodendrocyte transcription factors derived from a promoter-based analysis of co-expressed genes in myelinated mouse tissue.

To strengthen the association of genes in these lists with specific astrocyte, oligodendrocyte, or microglia functioning, genes were removed if found in more than one of these cell-types or if present in a curated exclusion list of general neuronal genes[5]. An enrichment analysis using GO biological processes was performed on the final, filtered astrocyte, oligodendrocyte, microglia and neuronal lists to see if processes associated with specific cell-type functions were uniquely enriched within each list. For each glial cell-type (oligodendrocytes, astrocytes, and microglia), functional gene-sets were created based on GO biological process annotations. Importantly, gene-sets were built according to the hierarchical structure of GO (i.e., higher-level parental nodes were subdivided into more specific child nodes), resulting in an organization of related gene-sets over a maximum of three levels and substantial overlap of genes between gene-sets[5].

For the present study few adaptions were made to the sets as described in Goudriaan et al.[5] in order to reduce the number of oligodendrocyte functional gene-sets and reduce multiple testing. Most importantly, gene-sets were grouped together into fewer, overarching functional sets (See Table 1). Specifically, the metabolism sets were grouped together into ‘metabolism related genes’, the cell signaling groups

into 'cell communication related genes', the intracellular sets into 'cell process related genes', and the cell development and immune system sets into a set of 'cell development & health related genes'.

Genes that were not annotated into gene-ontology (GO) biological processes were grouped together in a 'miscellaneous oligodendrocyte set'. Sets of genes regarded to be especially important for oligodendrocyte myelination related processes were kept in separate gene-sets, and the myelination and node genes were not added to the general group of 'cell process related genes', but included as a set of 'oligodendrocyte specific processes'.

In addition, in a secondary analyses we included the *NRG1* gene to the myelination gene-set because *NRG1* gene has well-documented roles in myelination in animal models[20-22], and others have also included the NRG1 gene in previous analyses of myelination genes and white matter integrity in schizophrenia[23]. In the glial gene-sets reported by Goudriaan et al.[5] this gene was not included (as it is believed to be axonally expressed), and Goudriaan et al.[5] paper's intended to focus on cell-type specific functions.

As our main goal was to test the role of genes involved in myelination (including genes that regulate expression of genes in oligodendrocytes but are not necessarily expressed in oligodendrocytes themselves), we show results for cell-type specific gene-sets as well as an expanded gene-set including the *NRG1* gene as a known regulator of myelination processes[20-22].

Participants

The sample used for this study has been described in detail elsewhere[24]. In summary, the curated DTI sample consisted of 114 subjects with schizophrenia, and 138 controls with available genetic data. Subjects were recruited from four sites: Massachusetts General Hospital in Boston (MGH) [N = 60; 29 cases, and 31 controls], University of Iowa (UI) [N = 92; 38 cases, and 54 controls], University of Minnesota (UMN) [N = 53; 25 cases, and 28 controls], and University of New Mexico (UNM) [N = 47; 22 cases, and 25 controls]. Healthy volunteers were recruited from the community,

the healthy control subjects were matched within site to the patient cohort for age, sex, handedness and parental education. Controls were excluded from the study if they had any Axis I psychiatric disorder, including substance abuse/dependence or a history of a schizophrenia or bipolar spectrum disorder in a first-degree relative. Additional exclusion criteria for both patients and controls included a neurological disorder affecting brain function (i.e., head injury with loss of consciousness and seizure disorder) or active substance abuse/dependence. Written informed consent was obtained from all subjects prior to participation, and the institutional review boards at each of the four sites approved the study. All the clinical, and imaging data used in this study is publically available through the neuroinformatics suite COINS (Collaborative Informatics Neuroimaging Suite) at www.coins.mrn.org.

Imaging

The image acquisition protocols used for this study have also been described previously[24]. In summary, structural MRI data were acquired with either a Siemens 1.5-Tesla (MGH, UI, and UNM) or a Siemens 3-Tesla (UMN) MR scanner. The T_1-weighted structural brain scans at each of the four sites were acquired with an in-plane resolution of 0.625×0.625 mm^2, a slice thickness of 1.5mm, and a flip angle of 7 degrees. MGH and UNM used a Siemens 1.5-Tesla scanner with repetition time (TR) = 12 ms, echo time (TE) = 4.76 ms, and number of excitations (NEX) = 1. UI used a GE 1.5-Tesla Genesis Sigma scanner with TR = 20 ms, TE = 6 ms, and NEX = 3. UMN used a Siemens 3-Tesla scanner with TR = 2530 ms, inverse time (TI) = 1100 ms, TE = 3.79 ms, and NEX = 1.

All diffusion tensor images (DTI) were obtained at each site with a 2mm isotropic resolution. MGH used a Siemens Sonata 1.5-Tesla scanner with TR = 8900 ms, TE = 80 ms, B values of 0 and 700, NEX = 1, and 60 directions. UI used a Siemens TRIO 3-Tesla scanner with TR = 9500 ms, TE = 90 ms, B values of 0 and 1000, NEX = 4 and 6 directions. UNM used a Siemens Sonata 1.5-Tesla scanner with TR = 9800 ms, TE = 86 ms, B values of 0 and 1000, NEX = 4 and 12 directions. UMN used a Siemens TRIO 3-Tesla scanner with TR = 10 500 ms, TE = 86 ms, B values of 0 and 1000, NEX = 2 and 12 directions.

The diffusion-weighted images were analyzed using the GTRACT [25]. Scalar measures for FA were calculated on the DTI images for all subjects, measurements of FA were calculated in coronal Talairach sections from the anterior to the posterior region along the whole brain. The mean FA within each coronal slice for all subjects were calculated, and a within-site z-transformation was performed prior to pooling the data, this is a crucial step as each site had large FA differences, so standardization was done prior to pooling the data in order to control for those differences.

In addition to obtaining coronal slices, regions from the Johns Hopkins University WM atlas (http://www.dtiatlas.org/) were applied to the FA maps to extract mean FA values for each individual, as this atlas selects only the major WM tracts, and evaluating FA within the mask provides a global mean DTI value of the major WM tracts.

Genotyping

Whole blood was collected from subjects for DNA extraction; whole-genome genotyping (1 million SNP's) was done using HumanOmni1 Quad Beadchip Kits (Illumina, San Diego). Genotyping data quality control (QC) was performed in order to assess the failure rate per individual and per SNP, the degree of relatedness between individuals, and to identify ancestral outliers, following the standard protocol for data quality control in genetic case-control association studies by Anderson et al.[26].

We removed 37 cases and 34 controls in the QC process.The vast majority of subjects that were removed from the study failed ancestry clustering; after removing these subjects the genomic inflation factor (based on median chi-squared) was 1.0153, and the mean chi-squared statistic is 1.0047. After QC 710224 SNPs remained for association analysis (the total genotyping rate in remaining individuals was 0.9989).

As previously published[24], due to consortium agreements and IRB restrictions, the raw genotypic data used in the study is not publically available, but GWAS results obtained as part of the gene-set analysis are publically available at GWAS Central: http://www.gwascentral.org/study/HGVST1829.

Statistics

The gene set association analyses were performed using a statistical software package developed by our group: the *Joint Association of Genetic Variants* (JAG) software (http://ctglab.nl/software/), the details of the statistical methods used by this software were described else where[27]. In summary, all SNPs that survived quality control were mapped to genes on the basis of NCBI (National Center for Biotechnology Information) human assembly build 36.3 and dbSNP release 129. For the definition of the gene boundaries we downloaded the 'seq_gene.md' file from the FTP website of NCBI. From this list of records we deleted genes coded as *pseudo* in the column 'feature_type'. Subsequently, we selected the records with *gene* as 'feature_type' and *reference* as 'group_label'. For these records, we assigned SNPs to genes when annotated between 'chr_start' (transcription start site) and 'chr_stop' (transcription stop site). We then conducted SNP association analyses using additive models of allele counts. Cochran–Mantel–Haenszel tests implemented in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/)[28] were used for the association analyses. These single SNP outcomes were then used to conduct a self-contained test by summing the logarithm of the reciprocal of the *p*-values (denoted as $\Sigma\text{-}\log_{10}(p)$ method) and permutation was used to determine the significance of the combined effect of all SNPs in a gene-set. For gene-sets with a significant empirical *p*-value we conducted competitive tests, to evaluate whether the gene-set was more significantly associated with the trait than 500 randomly composed, matched control gene-sets.

4.4 RESULTS

Descriptive

After removing subjects that failed ancestry clustering, the total sampled consisted of 181 subjects: 77 cases (76% male, mean age 35.65 [18-60]), and 104 controls (57% male, mean age 32.92 [19-58]). After removing subjects that didn't survive DTI quality control, total brain FA was available for 129 subjects: 48 cases, and 81 controls. In this subsample total brain FA was significantly decreased in cases compared to controls (ANOVA F= 15.6, $p = 0.0001$), which compares well to results reported previously for the entire sample[14].

Single SNP association analysis

SNP association analyses using additive models of allele counts were calculated in order to generate the association *p*-values needed for the gene set association analyses; a genome wide association analysis (GWAS) was performed in the final curated sample. As expected, we found that no single SNP reached genome wide significant association for schizophrenia (lowest $p = 5.195e-06$ for SNP rs2028122 on chromosome 15), or for FA (lowest $p = 4.08e-06$ for SNP rs1422121 on chromosome 5).Additionally, when looking a the individual P values for each SNP included in each oligodendrocyte gene set (data not shown), none of the SNP survived multiple testing correction for the numbers of SNPs in each gene set (see number of SNPs per gene set in Table 1).

Gene set association analysis

Based on our prior knowledge of schizophrenia being associated with glial gene-sets[5], and in order to reduce the number of tests and avoid multiple testing errors, we first analyzed whether any of the 6 primary oligodendrocyte gene-sets were associated with schizophrenia in our sample. We found that only the gene-set of oligodendrocyte-specific-functions was nominally associated with schizophrenia (N= 181, self-contained test $p = 0.04$; and competitive test $p = 0.05$) [Table 1].

The oligodendrocyte-specific-functions gene-set was divided into two subgroups as previously described by Goudriaan et al.[5]: i) the myelination gene-set, and ii) the node processing gene-set. Secondly, we tested whether any of these two gene-sets were also associated with schizophrenia; we found that the myelination gene-set was significantly associated with Schizophrenia (N = 181, self-contained test $p = 0.004$; and competitive test $p = 0.006$; both p-values surviving multiple testing correction); additionally, we performed the same analysis including only the subjects for which FA was available (N = 129), when found that in this smaller group, also the myelination gene-set was significantly associated with Schizophrenia using the competitive test ($p = 0.006$), but not with the self-contained test ($p = 0.06$). Third, we tested if the myelination gene-set that was significantly associated with schizophrenia was also associated with FA; the results showed no association of the myelin gene-set with FA (N = 129, self-contained test $p = 0.2$) [Table 2].

Table 1

Gene-set association analysis of the oligodendrocyte gene sets with schizophrenia.

Oligodendrocyte Gene Sets	Genes	SNPs	Self-Contained Test *P* Value
Oligodendrocytes ALL GENES	1893	42692	0.84
Subgroup 1: Metabolism	544	9979	0.76
Subgroup 2: Cell communication	281	8234	0.50
Subgroup 3: Cell processes	434	11278	0.83
Subgroup 4: Cell development & health	141	2638	0.49
Subgroup 5: Oligodendrocyte specific functions	24	908	**0.04**
5.1 Myelination	17	222	**0.004**
5.2 Node Processing	8	399	0.97
Subgroup 6: Miscellaneous	469	9655	0.52

Table 2

Gene-set association analysis of the myelination gene-set with schizophrenia and total brain fractional anisotropy (FA).

		Glial myelination gene-set		Glial myelination gene-set + *NRG1*	
	N	**Self-Contained Test *P* value**	**Comp Test *P* Value**	**Self-Contained Test *P* value**	**Comp Test *P* Value**
Schizophrenia	181	**0.007**	**0.005**	**0.001**	**0.001**
FA available §	129	0.061	**0.043**	**0.005**	**0.002**
Schizophrenia (FA as covariant)	129	**0.049**	**0.038**	**0.012**	**0.008**
Total brain FA	129	0.201	N/A	**0.040**	**0.024**
Total brain FA (SZ as covariant)	129	0.279	N/A	0.146	N/A

§ Association analysis including only subjects for which FA was available. Comp: Competitive.

As we did not want to limit ourselves to only one cell-type (i.e. only looking at genes predominantly expressed in oligodendrocytes), in a secondary analysis, we added a known regulator of myelination (the *NRG1* gene) to the myelination gene-set, which itself is not expressed in oligodendrocytes.

Rerunning our gene-set analyses for schizophrenia and FA showed that the expanded myelination gene-set was again significantly associated with schizophrenia (N = 181, self-contained test $p = 0.001$; and competitive test $p = 0.001$), this was also the case for the smaller sample including only the subjects with FA data available (N = 129, self-contained test $p = 0.005$; and competitive test $p = 0.002$), and now also with total brain FA (self-contained test $p = 0.04$; and competitive test $p = 0.024$) [Table 2].

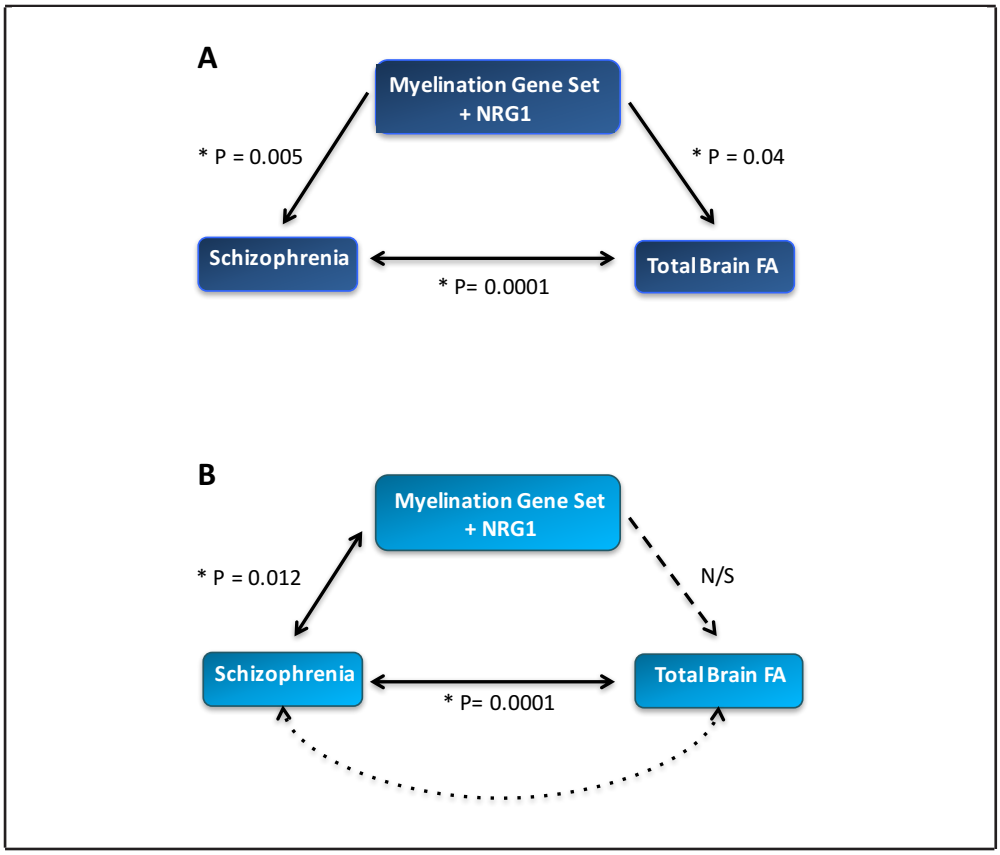

Figure 1. Combine effect of genetic variants of myelination genes is associated with schizophrenia and decreased total brain FA.

Model A shows how the myelination gene-set may explain part of the link between total brain FA and Schizophrenia, under this model it's not possible to establish the direction of causation, as is not possible to determine whether genetic variants in the myelination gene-set impact on FA which in turn increases the risk for SZ or vice versa.

Model B shows the effect when testing the association between the myelination gene-set and schizophrenia while correcting for total brain FA, and vice versa (denoted by the doted line). Under this model is possible to identify a possible direction of causation; while correcting for FA the association between the myelination gene-set and schizophrenia remains significant, but the association between the myelination gene set and total brain FA while correcting for schizophrenia is no longer significant (discontinuous line) indicating that the association between the myelination gene-set and total brain FA is mediated by schizophrenia diagnosis status.

N/S: No significant; FA: Fractional Anisotropy.

Gene-based analyses of all genes in the myelination gene set

The final myelination gene-set was composed of 18 genes Oligodendrocyte expressed genes and *NRG1* gene (Table 3). As the genotyping platform did not include any SNPs for the *POU3F1* gene, this gene was not included in any of the analyses. We used JAG to perform a gene-based association analysis of all genes included in the myelination gene-set to investigate whether one of these genes was independently associated with schizophrenia or total FA and was driving the gene-set association. We found nominal weak associations of *MOBP* and *OMG* with schizophrenia, *MAG* with FA, and *NRG1* with both schizophrenia and FA.

None of these associations would have survived multiple testing correction for the number of gene-based tests performed suggesting that the association of the gene-set with schizophrenia and FA was not driven by singleton genes, but instead indicating that the association was due to the combined effect of multiple genes within the gene-set.

Table 3

Results of gene based association analysis of myelination genes with schizophrenia and total brain fractional anisotropy.

Gene	SNPs	Gene Based Self-Contained Test Nominal *P* values	
		Schizophrenia	Total Brain FA
C11orf9	13	0.162	0.258
CNP	4	0.491	0.708
ILK	5	0.520	0.559
MAG	9	0.127	0.018
MAL	3	0.372	0.119
MBP	97	0.081	0.320
MOBP	24	0.007	0.784
MOG	11	0.056	0.842
OMG	1	0.027	0.959
CLDN11	4	0.928	0.341
PLP1	1	0.696	0.452
POU3F1	0	N/A	N/A
KLK6	2	0.239	0.941
TF	24	0.942	0.167
EIF2AK3	11	0.869	0.711
GAL3ST1	3	0.111	0.944
OLIG2	3	0.931	0.720
PLLP	7	0.264	0.383
NRG1	300	0.012	0.041

4.5 DISCUSSION

Global white matter integrity alterations have been consistently found in patients with schizophrenia[12; 29]. Whereas the cause of these alterations is still unknown, there is significant evidence for heritability of white matter integrity of specific brain regions in subjects with schizophrenia and their unaffected relatives[16-18]. These findings suggest that white matter integrity measures may be useful endophenotypes for genetic studies of schizophrenia[13].

To date, none of the significant genome-wide genetic variations associated with schizophrenia have been found to explain the differences in WM integrity seen in schizophrenia patients[30]. Individual genetic variants associated with schizophrenia have small effect sizes[3]. Thus, it has been suggested that the additive effect of multiple genetics variants with small effects may explain complex phenotypes[31]. Neuroimaging-based intermediate phenotypes have emerged as particularly promising because they map risk associated gene effects onto physiological processes in brain that are altered in patients[32]. In this study we sought to investigate whether glial gene-sets associated with schizophrenia were also associated with white matter integrity.

As previously reported by White et al.[14], FA was significantly decreased in schizophrenic subjects compared to controls in the same sample used for this study. White matter integrity abnormalities have been found in first episode antipsychotic-naïve schizophrenic patients[33], and are not believed to be a consequence of the disease itself or the antipsychotic treatment[34]. One possible mechanism of FA decrease involves an alteration or weakening of the myelin sheath. This is supported by histopathological studies suggesting a disturbed myelination in schizophrenia[35].

In our study we tested the combined effects of all genetic variants available within oligodendrocyte-expressed genes grouped in functional gene-sets. As in many other imaging genetic studies, our study was limited by sample size, specially after performing genetic quality control we had to remove 37 cases and 34 controls due to population stratification issues. From the 6 gene-sets tested, only the oligodendrocyte-specific-function gene-set was associated with schizophrenia; this gene set is composed of two separated sub gene-sets, of which only the myelination gene-set was significantly associated with schizophrenia, but not with FA.

In our previous, larger scaled study[5], we tested for association of 96 astrocyte, oligodendrocyte and microglia gene-sets with schizophrenia. We found an association of six astrocyte gene-sets and three oligodendrocyte gene-sets. The oligodendrocyte gene-sets included lipid metabolism, oxidation-reduction and gene transcription, yet the myelination gene-set did not survive multiple testing in Goudriaan et al.[5] although this gene-set showed nominal significance (uncorrected $p = 0.0203$).

A recent study by Voineskos et al.[23] tested whether individual genetic variants in myelin genes had an effect on white matter integrity. They found that one SNP located in the *MAG* gene (SNP rs756796) and one SNP in the *CNP* gene (SNP rs2070106) had an effect on the microstructural integrity of all white matter tracts. In our study, using a gene-based analysis, we did not find that any of the genes in our original myelination gene-set (including *MAG* and *CNP*) was individually significantly associated with schizophrenia, or total brain FA. Voineskos et al.[23] also included *NRG1* in their analysis of gene variants for myelination, as the myelin genes show epistatic risk for schizophrenia with variants in the *NRG1* gene system; expression of these two gene systems is coordinated[36], and disruption of the *Nrg1–ErbB4* pathway in oligodendrocytes in animal models leads to alteration of the myelin sheath of major white matter tracts, reduced conduction velocity, and cognitive changes[37].

Therefore, we conducted secondary analyses following Voineskos et al.[23], by including the *NRG1* gene in the myelin gene-set. We found that this expanded gene-set was again significantly associated with schizophrenia and now also with total brain FA, even though the *NRG1* gene by itself was only marginally associated with FA. This finding suggests that *NRG1* could modulate white matter integrity in the context of the additive effect seen with other myelin genes. Under this model, neuronally expressed *NRG1* likely regulates myelination genes through an effect on oligodendrocyte-expressed specific genes cells.

Altogether, we found a statistically significant association between schizophrenia and FA (confirming previously established links[11-14]), between the myelination gene-set and schizophrenia (replicating earlier, independent findings[5]), and between the myelination gene-set and FA (novel finding). Additionally, we tested for the association between the myelination gene-set and schizophrenia while correcting for FA, this association remains significant, indicating that the variance in schizophrenia liability

is partially explained by the myelin gene-set independently of the variance explained by FA. In the other hand, when testing the association between the myelination gene-set and FA while correcting for schizophrenia status; this association was no longer statistically significant, suggesting that the variance observed in total brain FA is explained only in part by the myelination gene-set, which indicates that there are other genetics variants associated with schizophrenia have an effect on FA that are not included in the myelination gene-set.

In conclusion, our findings support the hypothesis that multiple genetic variants in myelination-related genes contribute to the observed correlation between schizophrenia and decreased white matter integrity as measured by FA. These findings warrant further research in using myelination-related proteins as pharmaceutical targets for preventing white matter integrity loss as seen in schizophrenia.

4.6 ACKNOWLEDGMENTS

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Chapter 5

Schizophrenia Polygenic Risk and White Matter Integrity

CHAPTER 5

SCHIZOPHRENIA POLYGENIC RISK AND WHITE MATTER INTEGRITY[5]

5.1 ABSTRACT

White matter integrity (measured as total brain fractional anisotropy) has been consistently found to be decreased in subjects with schizophrenia. Both schizophrenia and fractional anisotropy are highly heritable traits, but it is unclear how white matter integrity abnormalities relate to the underlying genetic architecture of schizophrenia. We sought to investigate whether polygenic risk profiles for schizophrenia explain part of the variance of white matter integrity.

We used the Psychiatric Genomics Consortium Schizophrenia 2 (PGC-SCZ2) sample (36,989 cases and 113,075 controls) to calculate polygenic risk scores for each individual in an independent target sample of 80 subjects with schizophrenia, and 105 controls. We found that higher PGC-SCZ2-derived polygenic risk scores are significantly associated with decreased total brain fractional anisotropy in the total target sample (N= 185), explaining 3% of the variance of fractional anisotropy (P = 0.01); as well separately in cases (2% of variance explained, P = 0.04), and controls (3.3% of variance explained, P = 0.02).

In conclusion, polygenic risk scores for schizophrenia are significantly associated with decreased white matter integrity in subjects with schizophrenia and healthy controls; these findings suggest that structural connectivity of white matter tracts are genetically associated with schizophrenia. This finding was not limited to one specific brain region, suggesting a diffuse effect of schizophrenia-associated genetic variants on white matter integrity; and it's specific to white matter integrity, as none of the polygenic scores were found to be associated with total brain, white matter volume, gray matter volume, or lateral ventricles volume.

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5.2 INTRODUCTION

Although schizophrenia has a substantial heritable component (Lichtenstein et al., 2006), and a number of robust genetic variants have recently been identified (Fromer et al., 2014; Purcell et al., 2014; Ripke, 2014), the underlying neurobiology of this chronic disorder remains elusive.

It has been suggested that a disturbance in connectivity between different brain regions is responsible for the clinical symptoms of schizophrenia (Meyer-Lindenberg et al., 2005). Fractional anisotropy (FA) as measured by diffusion tensor magnetic resonance imaging (DTI) provides the directional information of local neuronal fibers, and has been used to estimate the neuroanatomical connectivity in the cerebral white matter (Kumazawa et al., 2006).

FA is a highly heritable trait for both the whole-brain, and for many individual brain regions (Skudlarski et al., 2013). Disruptions in white matter tract structures of various brain regions have been consistently implicated in the pathophysiology of schizophrenia (Friedman et al., 2008; Liu et al., 2013; Nakamura et al., 2012; Quan et al., 2013; Samartzis et al., 2013); although there is a lack of consistency in the spatial localization of the brain regions showing lower FA compared to healthy controls (White et al., 2008), several other studies have found that white matter abnormalities are not localized to a specific brain region, but instead reflect a diffuse process with widely dispersed focal reductions in FA that vary spatially among individuals (Ellison-Wright et al., 2014; Lee et al., 2013; White et al., 2011). In addition, white matter disruptions are present early in the course of schizophrenia (Szeszko et al., 2005), including in childhood-onset schizophrenia (Moran et al., 2015), suggesting that low FA may be an early marker of the disorder.

Polygenic risk scores have been successfully used as a predictor of both adult-onset, and childhood-onset schizophrenia (Ahn et al., 2014; Ripke, 2014). This approach has also been successfully used to investigate the effect of polygenic risk scores for schizophrenia on brain measures associated with schizophrenia; Terwisscha van Scheltinga et al. (Terwisscha van Scheltinga et al., 2013) found that polygenic risk scores for schizophrenia are significantly associated with total brain volume and white matter volume equally in subjects with schizophrenia and controls. Their findings suggested that a small subset of genetic risk variants is related to the development of

white matter, and that disruptions in white matter growth increase the susceptibility to develop schizophrenia. However, their study did not include a measure of white matter integrity, and a larger follow up study failed to replicate these findings (Papiol et al., 2014).

The aim of the current study is to determine to what extent polygenic risk for schizophrenia is related to brain white matter integrity. To the best of our knowledge this is the first study looking at the effect of polygenic risk scores for schizophrenia on white matter integrity. Determining the effects of an overall polygenic risk profile on white matter integrity might help to understand how a genetic susceptibility for schizophrenia leads to the manifestation of disease.

5.3 MATERIALS AND METHODS

Study Participants

The target sample used for this study is part of the MIND Clinical Imaging Consortium (MCIC) project, a multi-institutional study of first-episode and chronic schizophrenia patients. For our study, we selected all subjects for which genotypic, and diffusion tensor imaging (DTI) data were available. The final sample consisted of 80 subjects with schizophrenia, and 105 controls (distribution of cases and control per study site, and demographics of combined sample in Table 1).

The details of the MCIC sample have been described previously (Gollub et al., 2013). In summary, the subjects were recruited from four sites: Massachusetts General Hospital in Boston (MGH), University of Iowa (UI), University of Minnesota (UMN), and University of New Mexico (UNM). Healthy volunteers were recruited from the community; the healthy control subjects were matched within site to the patient cohort for age, sex, handedness and parental education. Controls were excluded from the study if they had any Axis I psychiatric disorder, including substance abuse/dependence or a history of a schizophrenia or bipolar spectrum disorder in a first-degree relative.

Table 1

Demographic Information

	SCZ Patients	Healthy Controls	Significance *P*
Subjects per Study Site:			
MGH	22	20	
UI	15	45	
UMN	23	17	
UNM	20	23	
Combined Sample:	N = 80	N= 105	
Gender (M/F)	58/22	59/46	
Age (SD)	34.9 (11.5)	32.3 (10.7)	0.98
Height in cm (SD)	172 (9.4)	172.8 (10.6)	0.58
Level of Education in years (SD)	13.12 (2.70)	14.4 (2.0)	<.001
Level of paternal education in years (SD)	14.16 (3.8)	14.98 (3.3)	0.14
Level of maternal education in years (SD)	13.46 (3.2)	14.01 (2.6)	0.22
Duration of illness in years (SD)	12.25 (11.14)	NA	
Duration of treatment in years (SD)	11.91 (11.15)	NA	
Intracranial Volume in mL (SD)	1662.3 (183.7)	1707.8 (228)	0.14
Total Brain Volume in mL (SD)	1058.4 (126.5)	1119.8 (123.5)	0.001
Total Brain FA (SD)	0.252 (0.028)	0.274 (0.027)	<.001

SCZ: Schizophrenia. MGH: Massachusetts General Hospital. UI: University of Iowa. UMN: University of Minnesota. UNM: University of New Mexico (UNM). SD: Standard Deviation. M/F: male/female.

Additional exclusion criteria for both patients and controls included a neurological disorder affecting brain function (i.e., head injury with loss of consciousness and seizure disorder) or active substance abuse/dependence. Written informed consent was obtained from all subjects prior to participation, and the institutional review boards at each of the four sites approved the study.

Clinical and imaging data from three sites used in this study are publically available through the neuroinformatics suite COINS (Collaborative Informatics Neuroimaging Suite) at www.coins.mrn.org.

Imaging

The image acquisition protocols used for this study have also been described previously (Gollub et al., 2013). In summary, structural MRI data were acquired with either a Siemens 1.5-Tesla (in three sites: MGH, UI, and UNM) or a Siemens 3-Tesla (in one site: UMN) MR scanner. The T_1-weighted structural brain scans at each of the four sites were acquired with an in-plane resolution of 0.625 × 0.625 mm^2, a slice thickness of 1.5mm, and a flip angle of 7 degrees. MGH and UNM used a Siemens 1.5-Tesla scanner with repetition time (TR) = 12 ms, echo time (TE) = 4.76 ms, and number of excitations (NEX) = 1. UI used a GE 1.5-Tesla Genesis Sigma scanner with TR = 20 ms, TE = 6 ms, and NEX = 3. UMN used a Siemens 3-Tesla scanner with TR = 2530 ms, inverse time (TI) = 1100 ms, TE = 3.79 ms, and NEX = 1. Cross site MRI acquisition calibration and reliability were established in a preceding study using human phantoms, following guidelines developed by the biomedical informatics research network (BIRN) test bed for morphometry (Jovicich et al., 2006; Jovicich et al., 2009). MCIC structural MRI data from three consecutive volumes were registered, motion corrected, averaged and analyzed in an automated manner with atlas-based FreeSurfer software suite (http://surfer.nmr.mgh.harvard.edu, Version 4.0.1).

All diffusion tensor images (DTI) were obtained at each site with a 2mm isotropic resolution. MGH used a Siemens Sonata 1.5-Tesla scanner with TR = 8900 ms, TE = 80 ms, B values of 0 and 700, NEX = 1, and 60 directions. UI used a Siemens TRIO 3-Tesla scanner with TR = 9500 ms, TE = 90 ms, B values of 0 and 1000, NEX = 4 and 6 directions. UNM used a Siemens Sonata 1.5-Tesla scanner with TR = 9800 ms, TE = 86 ms, B values of 0 and 1000, NEX = 4 and 12 directions. UMN used a Siemens TRIO 3-Tesla scanner with TR = 10 500 ms, TE = 86 ms, B values of 0 and 1000, NEX = 2 and 12 directions. The diffusion-weighted images were analyzed using the GTRACT (Cheng et al., 2006). Scalar measures for FA were calculated on the DTI images for all subjects and white matter regions were defined by the intersection between white matter segmentation from the structural MRI and FA greater than 0.1.

Global and regional FA were calculated within Talairach regions, which included the frontal, parietal, temporal, and occipital lobes, and the cerebellar and brain stem white matter, similar to our prior study (White et al., 2009).

The mean FA within each region was calculated, and a within-site z-transformation was performed prior to pooling the data, this is a crucial step as each site had large FA differences, so standardization was done prior to pooling the data in order to control for those differences. In addition to obtaining coronal slices, regions from the Johns Hopkins University WM atlas (http://www.dtiatlas.org/) were applied to the FA maps to extract mean core FA values for each individual, as this atlas selects only the major WM tracts, and evaluating FA within the mask provides a global mean DTI value of the major WM tracts.

Genotyping

Whole blood was collected from subjects for DNA extraction; whole-genome genotyping (1 million SNP's) was done using HumanOmni1 Quad Beadchip Kits (Illumina, San Diego).
Genotyping data quality control (QC) was performed in order to assess the failure rate per individual and per SNP, the degree of relatedness between individuals, and to identify ancestral outliers, following the standard protocol for data quality control in genetic case-control association studies by Anderson et al. (Anderson et al., 2010). After QC, 710224 SNPs remained for polygenic risk score analysis (the total genotyping rate in the individuals was 0.9989).

Polygenic Risk Scores Calculation

The discovery sample consisted of the "Schizophrenia 2" (SCZ2) dataset provided by the Psychiatric Genomics Consortium (PGC) (Ripke, 2014), which includes 36,989 cases and 113,075 controls. The polygenic risk profile training set was downloaded directly from http://www.med.unc.edu/pgc/downloads. The analysis only included the SNPs with minor allele frequency > 0.02 that were present in both the PGC-SCZ2 sample, and our independent target sample (the MCIC study is not part of the PGC dataset).

PGC-SCZ2-derived polygenic risk scores were calculated for each individual in the target sample (MCIC) using PLINK (Purcell et al., 2007). For each SNP, the number of risk alleles (0, 1, or 2) was weighted by the logarithm of the odds ratio observed for that particular SNP in the GWAS summary statistics of the PGC2 data. This outcome was then summed over all SNPs to obtain a polygenic risk score per individual. Multiple polygenic risk scores were defined which included different sets of SNPs based on nine *P*-value thresholds (P_T) in the discovery data (.001, .01, .05, .1, .2, .3, .4, .5, 1).

Statistical Analysis

In order to control for any possible large-scale differences in ancestry in the MCIC target sample, a principal component analysis (PCA) was conducted. In the PCA model of ancestry detection, the observations are the individuals and the potentially correlated variables are the markers, we calculated principal components using EIGENSTRAT (Anderson et al., 2010). We used the first two principal components after which the genomic inflation factor decreased from 1.7094 to 1.00 after correction. The two principal components were included as covariates in all subsequent statistical analysis involving genotypic data in order to control for any population stratification in the sample.

Also, in order to control for differences across sites, we included the imaging adquisition site as covariate for all the analyses. Linear (for continuous traits), and logistic (for dichotomous traits) regressions were calculated using SPSS (IBM SPSS Statistics for Macintosh. Version 22.0. Armonk, NY). A logistic regression analysis was used to estimate the variance explained in disease state by the PGC-SCZ2-derived polygenic risk scores in the target sample.

The variance explained by the scores was calculated as the difference in the Nagelkerke pseudo R^2 from a model including the score and covariates (principal components to control for population stratification, imaging site to control for differences across sites, and disease status) versus a model including only the covariates (without the score). Sex was used as a negative control of a dichotomous trait; we estimated the variance explained in sex by the score as the difference in the Nagelkerke pseudo R^2 from a model including the score and covariates versus a model including only the

covariates. Linear regression was used to estimate the variance explained in FA by the PGC-SCZ2-derived polygenic risk scores in the target population. The variance explained by the scores was calculated as the difference in the R^2 from a model including the score and covariates (principal components to control for population stratification, imaging site to control for differences across sites, and disease status) versus a model including only the covariates (without the score). Height was used as a negative control of a continuous trait; we estimated the variance explained in height by the score as the difference in the R^2 from a model including the score and covariates versus a model including only the covariates.

Additionally, we tested the variance explained by the PGC-SCZ2-derived polygenic risk scores in total brain volume, total grey matter volume, total white matter volume, and total lateral ventricles volume by the PGC-SCZ2-derived polygenic risk scores using the following covariates: intracranial volume (ICV) to control for head size, principal components to control for population stratification, imaging site to control for differences across sites, age, and disease status).

5.4 RESULTS

First, we confirmed the case/control differences in the white matter FA of the subsample as it was previously described for the complete MCIC sample (White et al., 2011). We found that in the MCIC sample the FA mean values for total brain (including frontal, temporal, parietal and occipital regions) were significantly reduced in subjects with schizophrenia compared to controls; this was not the case for the FA of the cerebellum and the brain stem, where no significant differences were found between cases and controls (Supplementary Table S1).

The MCIC sample is independent from the PGC-SCZ2 sample as none of the cases/controls were included in the PGC-SCZ2 dataset. As expected, we found that that the PGC-SCZ2-derived polygenic risk scores calculated using the target sample are significantly higher in cases compared to controls in our independent sample (Supplementary Table S2). We found that the PGC-SCZ2-derived polygenic risk scores strongly predict schizophrenia case/control status in the target sample, explaining ~15% of the variance ($P = 7.0 \times 10^{-6}$) [Figure 1, and Table 2].

We also found that the polygenic risk scores for schizophrenia were significantly associated with total brain FA in the complete target sample (cases and controls), and the association was stronger for increasingly liberal PT thresholds. The risk score on the basis of all the SNPs derived from the PGC-SCZ2 discovery sample PT < 1 was highly correlated with FA of the total brain in the target sample explaining ~3% of the variance (P = 0.01). The association remained significant after the splitting the sample in cases (2% of variance explained, P = 0.04), and controls (3.3% of variance explained, P = 0.02) [Figure 1, and Table 2].

We used height (for continuous traits), and gender (dichotomous traits) as negative controls, as we did not expect the variance of those two phenotypes to be explained by the PGC-SCZ2-derived polygenic scores. Our analyses did not show a significant prediction of polygenic risk for schizophrenia on gender or height (Figure 1, and Table 2).

After confirming that the PGC-SCZ2-derived polygenic scores were significantly associated with FA on the total brain; we tested whether the PGC-SCZ2-derived polygenic scores were also correlated with the FA of the different brain regions separately (frontal, temporal, parietal, and occipital regions). We found that except for the frontal region, the PGC-SCZ2-derived polygenic scores were highly correlated with the FA of all other brain regions, explaining 4.1% of the variance of FA in the temporal region (P = 0.004), 2.2% of the variance of FA in the parietal region (P = 0.02), and 3.6% of the variance of FA in the occipital region (P = 0.005) [Figure 2, and Table 3].

In a secondary analysis, in order to verify that our findings were specific to white matter integrity, and not to differences in brain volumes. We tested whether the PGC-SCZ2-derived polygenic scores were also correlated with total brain volume, total grey volume, total white volume, and total lateral ventricles volume. Our analyses did not show a significant prediction of polygenic risk for schizophrenia on volumetric brain measures (Table 4).

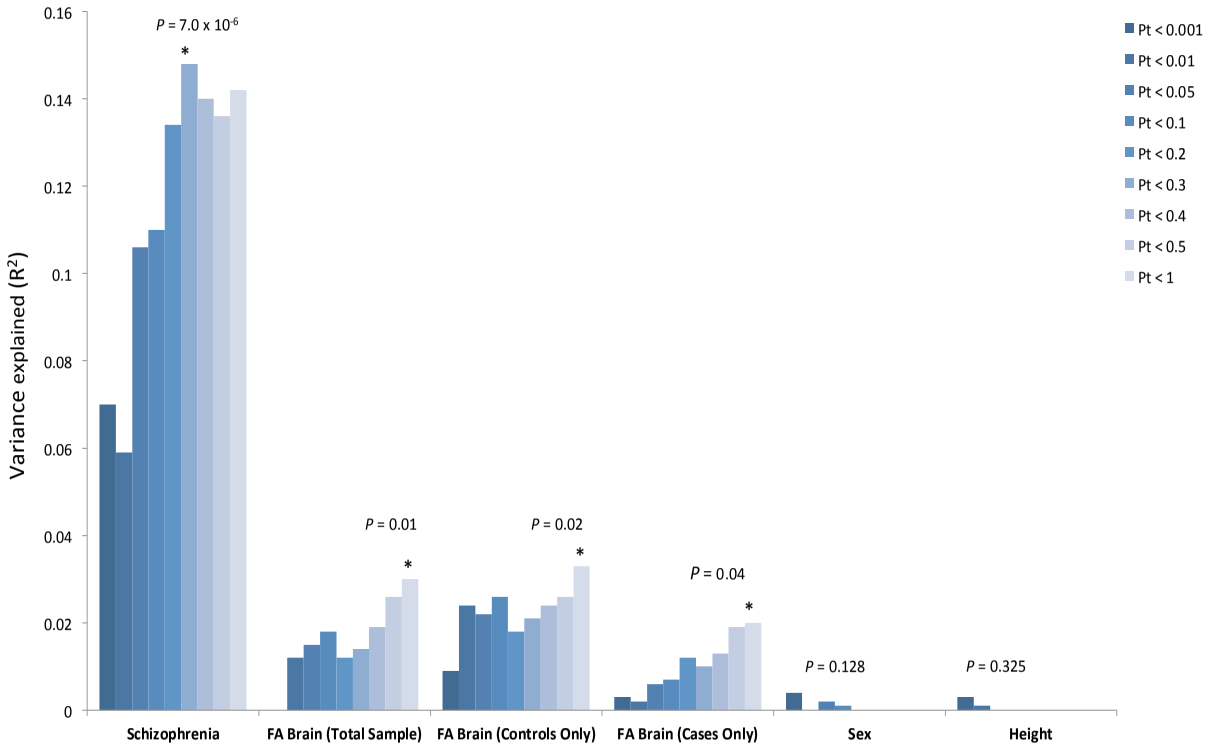

Figure 1. Variance explained by schizophrenia risk scores. Variance explained in the target sample on basis of the PGC2 schizophrenia risk score using nine significance thresholds (PT): <0.001, <0.01, <0.05, <0.1, <0.2, <0.3, <0.4, <0.5, <1.0). The y axis indicates the Nagelkerke's pseudo R2 for dichotomous traits (Schizophrenia and sex), and the R2 for continuous traits (FA of the total brain in complete sample, FA in controls only, FA in cases only, and Height). The number above each set of bars indicates P value for the highest variance explained by the PT denoted with * (for significant associations only). All analyses for FA were corrected for site of imaging acquisition, and population stratification; the analysis using the complete sample was also corrected for disease status as covariate in addition to those covariates.

Table 2

Variance explained of different phenotypes by schizophrenia polygenic risk scores.

	Schizophrenia N= 185		FA Brain (Total Sample) N= 185		FA Brain (Cases Only) N= 105		FA Brain (Controls Only) N= 80		Height N= 185		Sex N=185	
Risk Score	**Nagelkerke pseudo R^2**	***P***	**R^2**	***P***	**R^2**	***P***	**R^2**	***P***	**R^2**	***P***	**Nagelkerke pseudo R^2**	***P***
Pt < 0.001	0.07	**0.001**	0	0.99	0.009	0.32	0.003	0.63	0.003	0.12	0.004	0.32
Pt < 0.01	0.059	**0.003**	0.012	0.10	0.024	0.09	0.002	0.70	0.001	0.63	0	0.99
Pt < 0.05	0.106	**<.001**	0.015	0.06	0.022	0.11	0.006	0.51	0	0.76	0.002	0.60
Pt < 0.1	0.11	**<.001**	0.018	0.04	0.026	0.08	0.007	0.46	0	0.94	0.001	0.67
Pt < 0.2	0.134	**<.001**	0.012	0.10	0.018	0.15	0.012	0.35	0	0.79	0	0.75
Pt < 0.3	0.148	**<.001**	0.014	0.07	0.021	0.11	0.01	0.29	0	0.76	0	0.81
Pt < 0.4	0.14	**<.001**	0.019	**0.03**	0.024	0.08	0.013	0.17	0	0.83	0	0.74
Pt < 0.5	0.136	**<.001**	0.026	**0.01**	0.026	0.07	0.019	0.07	0	0.76	0	0.81
Pt < 1	0.142	**<.001**	0.03	**0.01**	0.033	**0.02**	0.02	**0.04**	0	0.76	0	0.74

All analyses were corrected for population stratification, and Site. Additionally, Total Brain Fractional Anisotropy of total sample, Height, and Sex were also corrected for disease status. Significant *P* values in bold. Pt: significance threshold. FA: fractional anisotropy.

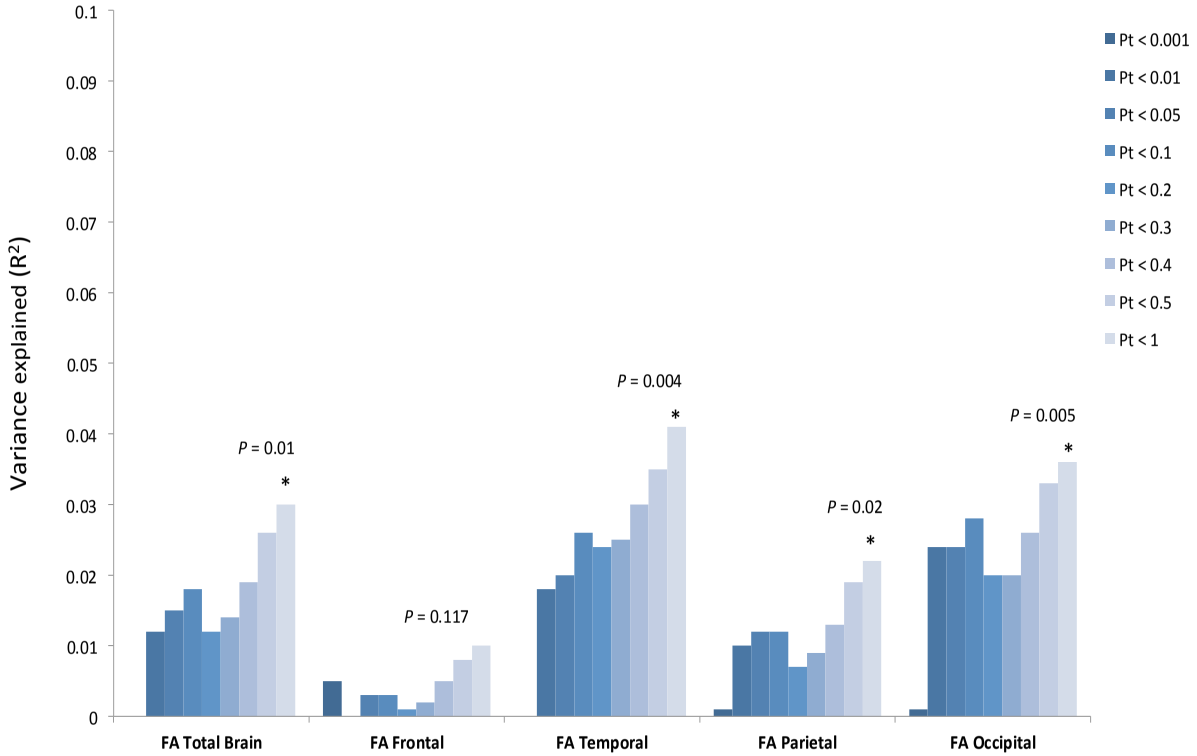

Figure 2. Variance explained of FA by schizophrenia risk scores. Variance explained in the target sample on basis of the PGC2 schizophrenia risk score using nine significance thresholds (PT <0.001, <0.01, <0.05, <0.1, <0.2, <0.3, <0.4, <0.5, <1.0). The y axis indicates the R2 for the FA of the total brain, and separately for the FA of the different brain regions: frontal, temporal, parietal, and occipital. The number above each set of bars indicates the P value for the PT < 1.0. All analyses for FA were corrected for disease status, site of imaging acquisition, and population stratification.

Table 3

Variance explained of fractional anisotropy by schizophrenia polygenic risk scores in different brain regions.
N=185

	FA Total Brain		FA Frontal Regions		FA Temporal Regions		FA Parietal Regions		FA Occipital Regions	
Risk Score	**R^2**	***P***	**R^2**	***P***	**R^2**	***P***	**R^2**	***P***	**R^2**	***P***
Pt < 0.001	0	0.99	0.005	0.3	0	0.93	0.001	0.75	0.001	0.56
Pt < 0.01	0.012	0.10	0	0.81	0.018	0.05	0.01	0.15	0.024	**0.02**
Pt < 0.05	0.015	0.06	0.003	0.41	0.02	**0.04**	0.012	0.11	0.024	**0.02**
Pt < 0.1	0.018	0.04	0.003	0.38	0.026	**0.02**	0.012	0.10	0.028	**0.01**
Pt < 0.2	0.012	0.10	0.001	0.67	0.024	**0.02**	0.007	0.24	0.02	**0.04**
Pt < 0.3	0.014	0.07	0.002	0.46	0.025	**0.02**	0.009	0.17	0.02	**0.03**
Pt < 0.4	0.019	**0.03**	0.005	0.27	0.03	**0.01**	0.013	0.09	0.026	**0.01**
Pt < 0.5	0.026	**0.01**	0.008	0.16	0.035	**<.001**	0.019	**0.04**	0.033	**<.001**
Pt < 1	0.03	**0.01**	0.01	0.11	0.041	**<.001**	0.022	**0.02**	0.036	**<.001**

All analyses were corrected for the following covariates: disease status, population stratification, and Site. Significant *P* values in bold. Pt: significance threshold.
FA: fractional anisotropy.

Table 4

Variance explained of different brain volumes
by schizophrenia polygenic risk scores.
N=185

Risk Score	Total Brain Volume		Cerebral Grey Matter Volume		Cerebral White Matter Volume		Ventricles Volume	
	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
Pt < 0.001	0.001	0.473	0	0.716	0.001	0.526	0.002	0.432
Pt < 0.01	0	0.908	0.001	0.443	0.001	0.584	0.012	0.063
Pt < 0.05	0.001	0.537	0.005	0.138	0.001	0.522	0.012	0.071
Pt < 0.1	0.002	0.298	0.006	0.107	0	0.679	0.012	0.069
Pt < 0.2	0.003	0.207	0.008	0.058	0	0.955	0.008	0.151
Pt < 0.3	0.004	0.092	0.011	0.053	0	0.802	0.012	0.103
Pt < 0.4	0.006	0.056	0.015	0.051	0	0.699	0.009	0.112
Pt < 0.5	0.006	0.059	0.015	0.059	0	0.751	0.013	0.095
Pt < 1	0.006	0.055	0.016	0.057	0	0.777	0.011	0.091

All analyses were corrected for the following covariates: Intracranial volume, age disease status, population stratification, and Site. Pt: significance threshold.

5.5 DISCUSSION

We set out to investigate whether polygenic risk scores for schizophrenia would explain a significant proportion of the variance of brain FA. Using the Psychiatric Genomics Consortium Schizophrenia-2 sample (36,989 cases and 113,075 controls), we calculated polygenic risk scores for each individual in an independent target sample of 80 subjects with schizophrenia, and 105 controls. We first confirmed that the polygenic risk scores for schizophrenia predicted the case/control status in our independent sample (Nagelkerke $R^2 = 0.15$); this result is similar to the one obtained by the recent study of the Schizophrenia PGC ($R^2 = 0.18$) (Ripke, 2014), indicating that the PGC-SCZ2-derived risk profile score is a consistent measure of schizophrenia risk (table 2).

Then we showed that white matter microstructure (as measured with FA) was associated with schizophrenia case-control status, and that the PGC-SCZ2-derived polygenic risk scores for schizophrenia were progressively correlated with FA of the total brain in the complete target sample, and separately in both cases and controls (table 2). Our findings are specific to white matter integrity only, as none of the polygenic scores were found to be associated with total brain, white matter volume, gray matter volume, or lateral ventricles volume (table 4); this is consistent with a recent study that failed to replicate an association between schizophrenia polygenic risk scores with total brain volume and white matter volume (Papiol et al., 2014).

Our finding that the PGC-SCZ2-derived polygenic risk scores for schizophrenia were progressively correlated with FA are not specific to a determined brain region, as the variance of the FA of different brain regions were equally explained by the schizophrenia risk scores when analyzed separately, except in the frontal lobe which did not show significant explained variance (table 3). Altogether, these findings suggest that there is a shared genetic link between schizophrenia and global white matter integrity. White matter integrity abnormalities are found as early as in child-onset schizophrenia (Moran et al., 2015), and in treatment-naïve patients (Lee et al., 2013), so it is unlikely that white matter abnormalities are a consequence of the disorder or an effect of the medications used to treat schizophrenia.

Although our results suggest a polygenic link between white matter integrity and schizophrenia, one of the limitations of this study is that it cannot pinpoint any specific candidate genes that could be shared between white matter integrity and schizophrenia. The polygenic risk score is mainly driven by the most significant genetic variants associated with schizophrenia, which were described in the recent GWAS study of schizophrenia by the PGC (Ripke, 2014). In that study the authors found that many of the genes that were significantly associated with schizophrenia are important for both neuronal and immune functions. Interestingly, in multiple sclerosis (MS) -a well-known autoimmune disorder that affects primarily white matter tracts- the white matter integrity is randomly decreased across different brain regions, and those changes in white matter integrity have been linked to cognitive deficits (spatial memory, information processing speed, verbal memory and learning, and working memory are impaired in patients with active MS) (Hulst et al., 2013); and those cognitive deficits are similar to those found in schizophrenic patients (Schaefer et al., 2013).

Our findings may in part support prior epidemiological studies proposing a role for an immune deregulation in schizophrenia (Benros et al., 2012), as immune-related genes could have an effect in white matter integrity in schizophrenia. Moreover, we also found recently that a set of myelination-related genes are significantly associated with both FA and Schizophrenia (Chavarria-Siles et al., 2015), suggesting a shared genetic component between white matter integrity and schizophrenia.

In conclusion, polygenic risk scores for schizophrenia can explain part of the inter-individual variation in white matter integrity, in both schizophrenia patients and healthy controls. This finding is not limited to one specific brain region, suggesting a diffuse effect of schizophrenia-associated genetic variants on white matter integrity. Although FA measurements currently cannot be used clinically to diagnose schizophrenia, a better understanding of the relationship between white matter integrity abnormalities and schizophrenia pathophysiology will likely translate into better and more specific treatments for this severe mental illness.

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5.8 SUPPLEMENTARY MATERIAL

Table S1

ANOVA of the means of the Fractional Anisotropy
per brain region, cerebellum, and brain stem.
(Cases = 80 / Controls =105)

Brain Region	**F**	***P***
FA total brain:	26.960	2.6×10^{-7}
FA frontal lobes	30.909	9.4×10^{-8}
FA temporal lobes	13.829	2.6×10^{-4}
FA parietal lobes	26.984	5.4×10^{-7}
FA occipital lobes	23.235	3.1×10^{-6}
FA cerebellum	1.763	0.186
FA brain stem	6.153	0.014

Table S2

ANCOVA of the means of the Schizophrenia polygenic risk scores
(Controlled for population stratification)
(Cases = 80 / Controls =105)

Schizophrenia Polygenic Risk Scores	**F**	**Significance *P***
$P_T < 0.001$	23.007	1.1×10^{-12}
$P_T < 0.01$	65.237	1.2×10^{-28}
$P_T < 0.05$	97.583	1.3×10^{-37}
$P_T < 0.1$	113.149	2.7×10^{-41}
$P_T < 0.2$	123.157	1.7×10^{-43}
$P_T < 0.3$	146.085	4.1×10^{-48}
$P_T < 0.4$	155.757	6.5×10^{-50}
$P_T < 0.5$	154.074	1.3×10^{-49}
$P_T < 1.0$	155.085	8.7×10^{-50}

Chapter 6

Genetic Link Between Genes Implicated in Schizophrenia and Subcortical Brain Structures

CHAPTER 6

GENETIC LINK BETWEEN GENES IMPLICATED IN SCHIZOPHRENIA AND SUBCORTICAL BRAIN STRUCTURES[6]

6.1 ABSTRACT

Subcortical brain volumes have been found to be significantly different in subjects with schizophrenia compared to healthy controls, yet the profile of brain structural abnormalities in schizophrenia is still not fully understood. In this study we sought to identify shared genetic variants between brain structure volumetric phenotypes and schizophrenia. We conducted a gene-set analysis to investigate whether gene sets previously implicated in schizophrenia are associated with a number of volumetric brain structures using publicly available results from a GWAS of eight brain measures of interest: intracranial volume (N= 11,373) and total bilateral volumes of the nucleus accumbens (N= 13,112), amygdala (N= 13,112), caudate nucleus (N= 13,171), hippocampus (N= 13,171), pallidum (N= 13,142), putamen (N= 13,145), and thalamus (N= 13,193).

We found that a gene-set of nuclear proteins previously implicated in schizophrenia was significantly associated with intracranial volume (competitive $p = 3.5x10^{-5}$), and we also found the microRNA-137 gene set was significantly associated with the total volume of the putamen (competitive test $p = 7.0x10^{-5}$).

Our findings suggest that there is a shared genetic component between specific gene-sets associated with schizophrenia and volumetric brain phenotypes. Understanding this genetic link may provide a better insight of the genetic mechanisms driving brain development and this severe psychiatric disorder.

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6.2 INTRODUCTION

Schizophrenia is a psychiatric disorder with substantial morbidity, mortality and personal and societal costs[1]. Schizophrenia is a highly heritable disorder[2] with a complex genetic etiology[3-5]; despite the robust number of genetic variants that have recently been associated with schizophrenia[5], the underlying neurobiology of this chronic disorder remains elusive.

The profile of brain structural abnormalities in schizophrenia is still not fully understood. A recent study identified several subcortical brain volumes that were significantly different in subjects with schizophrenia compared to healthy controls. Specifically it was found that subjects with schizophrenia had smaller hippocampus, amygdala, thalamus, accumbens, and intracranial volumes; as well as larger pallidum, and lateral ventricle volumes[6].

Convergent evidence from neuroimaging studies in schizophrenia suggests subtle but widespread gray matter reductions predominantly in the frontal and subcortical temporo–limbic regions[7]. These subcortical brain regions are involved in forming circuits with cortical areas to coordinate movement, learning, memory and motivation; and altered circuits can lead to abnormal behavior and disease, such as schizophrenia[8]. The heritability estimates for all subcortical brain regions are high, with the highest heritability estimates observed for the thalamus and caudate nucleus and lowest for the left nucleus accumbens[9].

In a recent large genome wide association analysis (GWAS) of magnetic resonance images of 30,717 individuals from 50 cohorts, several common genetic variants were reported that underlie variation in different structures within the human brain. Many of those genetic variants seem to exert their effects through known developmental pathways including apoptosis, axon guidance and vesicle transport; the strongest effects were found for the putamen and hippocampal volumes[7].

Although schizophrenia also having a substantial heritable component, it isn't clear whether the same (sets of) genes that are important for schizophrenia are also important for brain structural variation, and whether the differences in brain structures found in subjects with schizophrenia are genetically linked to the disorder.

A recent study integrating results from common variant studies of schizophrenia (33,636 cases, 43,008 controls) and volumes of several (mainly subcortical) brain structures (11,840 subjects) did not find evidence of genetic overlap between schizophrenia risk and subcortical volume measures either at the level of common variant genetic architecture or for single genetic markers[10].

Additionally, other studies looking for the effect of polygenic risk scores of schizophrenia on brain volume measures in healthy subjects reported contradicting results. Terwisscha van Scheltinga et al.[11] reported a decrease in total brain volume and white matter volume with increasing burden of risk variants for schizophrenia, whereas Papiol et al.[12] and van der Auwera et al.[13] found no association between schizophrenia risk scores with brain volumes in a healthy sample.

In this study we sought to investigate whether gene sets previously implicated in schizophrenia also play a role in volumetric brain structures. Gene-set analysis are statistical methods for analyzing multiple genetic markers simultaneously to determine their joint effect, these methods can be used when the effects of individual markers is too weak to detect, which is a common problem when studying polygenic traits[14]. Identifying shared genetic pathways between brain structure phenotypes and schizophrenia may provide a better insight of the pathophysiology of this neuropsychiatric dysfunction.

6.3 METHODS

Imaging and Genetics data

We used the publicly available data from the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium. We obtained directly from ENIGMA the p-values of a GWAS conducted as part of a collaborative large-scale genetic analysis of magnetic resonance imaging (MRI) scans to identify genetic variants that influence brain structure in healthy subjects. The sample population description, and detailed imaging methods have been previously published elsewhere[8]. Briefly, the brain measures examined in this study were obtained from structural MRI

data collected at participating sites around the world. Brain scans were processed and examined at each site locally, following a standardized protocol procedure to harmonize the analysis across sites.

The standardized protocols for image analysis and quality assurance are openly available online at http://enigma.ini.usc.edu/protocols/imaging-protocols/. The subcortical brain measures (nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus) were delineated in the brain using well-validated, freely available brain segmentation software packages: FIRST[15], part of the FMRIB Software Library (FSL), or FreeSurfer[16].

Genotyping and Genome Wide Association Analyses in ENIGMA data

The genotyping methods, as well as the GWAS results have been published elsewhere[8]. Briefly, each subject in the original ENIGMA study was genotyped using commercially available platforms. Before imputation, genetic homogeneity was assessed in each sample using multi-dimensional scaling (MDS) analysis, and ancestry outliers were excluded through visual inspection of the first two components. Quality control filtering was applied to remove genotyped single nucleotide polymorphisms (SNPs) with low minor allele frequency (< 0.01), poor genotype call rate (< 95%), and deviations from Hardy–Weinberg equilibrium ($P < 1 \times 10^{-6}$) before imputation.

The imputation protocols used MaCH[17] for haplotype phasing, and MINIMAC[18] for imputation, both protocols are freely available online at: http://enigma.ini.usc.edu/protocols/genetics-protocols/.

Genome-wide association scans were conducted at each site for all eight traits of interest including the ICV (N= 11,373) and total bilateral volumes of the nucleus accumbens (N= 13,112), amygdala (N= 13,112), caudate nucleus (N= 13,171), hippocampus (N= 13,171), pallidum (N= 13,142), putamen (N= 13,145), and thalamus (N= 13,193). For each SNP in the genome, the additive dosage value was regressed against the trait of interest separately using a multiple linear regression framework controlling for age, sex, 4 MDS components, and ICV (for non-ICV phenotypes).

Definition of gene sets

We included 33 expert-curated sets of genes (table 1) that have been previously implicated schizophrenia in other studies: these gene sets cover different sets of functionally related genes[19, 20], sets of co-expressed genes[21], sets representing protein complexes or networks[22-26], and genes extracted from genome-wide CNV studies, GWAS, and exome sequencing of de novo mutations[27].

Statistical Analyses

We conducted a gene-set analysis using MAGMA (Multi-marker Analysis of Genomic Annotation)[14], a freely available software for gene and gene-set analysis of GWAS genotype data (http://ctglab.nl/software/magma). MAGMA analysis can be performed on raw GWAS data, or on SNP p-values. For this study we used the p-values from the GWAS of different subcortical brain volumes and ICV provided by the ENIGMA consortium as described above. MAGMA allows gene association analyses using a multiple regression approach to properly incorporate linkage disequilibrium (LD) between markers and to detect multi-marker effects. The 1000 Genomes European panel was used as reference data set to account for LD between SNPs.

To perform the gene-set analysis, for each gene, the p-value computed in the gene analysis was converted to a Z-value. This yields a roughly normally distributed variable that reflects the strength of the association each gene has with the phenotype, with higher values corresponding to stronger associations.

MAGMA performs two separated analyses for each gene-set, 1) the self-contained gene-set analysis which tests whether the genes in a gene-set are jointly associated with the phenotype of interest; and 2) the competitive gene-set analysis which tests whether the genes in a gene-set are more strongly associated with the phenotype of interest than other genes. Both self-contained and competitive gene-set analyses are implemented using a gene-level regression model, a statistical method for analyzing multiple genetic markers simultaneously to determine their joint effect.

We consider a gene set significantly associated with a phenotype if the competitive test is significant after correction for multiple testing using Bonferroni correction for the number of tested gene sets as well as the number of phenotypes: the corrected significance level is thus 0.05/(33 gene-sets x 8 traits) = 0.0001.

6.4 RESULTS

We tested for association of the 33 gene sets previously implicated in Schizophrenia with each of the available brain volumes. We found evidence for significant association of two gene sets with at least one of the volumetric phenotypes (Table 1). First, we found that the gene set of *nuclear proteins* is significantly associated with ICV ($p = 3.5x10^{-5}$). This gene-set is composed of 157 genes.

Although none of the individual genes within the gene set was significantly associated with ICV after controlling for multiple testing; 4 genes showed strong association with ICV: *FAR1* (Gene ID: 84188) [p = 0.002], and *FADS1* (Gene ID: 3992) [p = 0.009] on chromosome 11; *OXCT1* (Gene ID: 5019) [p = 0.004] on chromosome 5, and GNL3 (Gene ID: 26354) [p = 0. 009] on chromosome 3 (Supplementary Table S1).

The remaining genes within this gene set did not have nominal significant P-values. The overall association of the gene-set was significantly stronger than expected under the competitive null hypothesis, suggesting that a single or few genes did not drive this association.

Second, we found that the gene set of "high confidence targets of microRNA-137" was significantly associated with the total volume of the putamen (competitive $p = 7.0x10^{-5}$); out of the 398 genes within this gene set, only the *DENND4B* gene (Gene ID: 9909) on chromosome 1 was individually significantly associated with putamen volume [p = 0.0001] (Supplementary Table S2), again, the overall association of the gene-set was significantly stronger than expected under the competitive null hypothesis, and a single or few genes did not drive this association.

6.5 DISCUSSION

Our aim was to investigate whether gene sets previously implicated in schizophrenia are associated with different volumetric brain structures. We performed a gene-set analysis using the results (*p* values) of a GWAS of a large brain imaging study of healthy subjects by the ENIGMA consortium. We tested for association between gene sets previously associated with schizophrenia and volumetric measures of intracranial volume, and seven different subcortical brain volumes (nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen, and thalamus); as these brain volumes have been previously found to be significantly different in subjects with schizophrenia when compared with healthy controls[5].

We tested a total of 33 expert-curated gene sets, and we found that the nuclear proteins gene set as defined by Kirov et al.[23] was significantly associated with ICV. When looking at the individual gene level, we found that none of the individual genes within this gene set was significantly associated with ICV; indicating that none of the genes was individually driving the association, but instead the gene set as a whole is associated with ICV (Supplementary table S1).

We also found that one of the candidate gene sets for schizophrenia described by Purcell et al.[27], the gene set composed by high confidence gene targets of the microRNA-137, was significantly associated with the volume of the putamen. We tested 3 different gene sets of microRNA-137 targets (see table1), these targets were selected by different authors using different methods[24,26,27], and all of them were previously implicated in schizophrenia.

It is important to notice that several gene sets have overlapping genes, and therefore not all gene sets can be consider independent; despite this dependency among gene sets, we decided to use a very stringent correction method for multiple testing (Bonferroni correction) to set a significance threshold for this study (0.0001).

Out of the three microRNA-137 gene sets, only the "high confidence targets of microRNA-137"[27] was significantly associated with putamen volume. Within this gene set, the *DENND4B* gene (Gene ID: 9909) showed a statistically significant association with putamen volume (Supplementary table S2).

Table 1.

Gene-set association analysis of sets of gene previously implicated in schizophrenia and intracranial brain volume and subcortical brain volumes.

Gene sets previously implicated in Schizophrenia	Reference	Genes N	ICV *P**	Accumbens *P**	Amygdala *P**	Caudate *P**	Hippocampus *P**	Pallidum *P**	Putamen *P**	Thalamus *P**
Schizophrenia GWAS (SNPs $P < 10^{-4}$)	Purcell et al., 2014[27]	401	0.176	0.218	0.158	0.268	0.884	0.922	0.016	0.255
Schizophrenia de novo CNV	Purcell et al., 2014[27]	144	0.263	0.301	0.733	0.245	0.958	0.407	0.025	0.391
Psychosis de novo CNV	Purcell et al., 2014[27]	175	0.554	0.818	0.870	0.435	0.764	0.711	0.783	0.204
Schizophrenia de novo disrup (exome sequencing)	Purcell et al., 2014[27]	80	0.050	0.919	0.566	0.489	0.306	0.726	0.066	0.523
Schizophrenia de novo nosyn (exome sequencing)	Purcell et al., 2014[27]	572	0.262	0.726	0.485	0.266	0.867	0.695	0.202	0.673
microRNA-137 (High confidence targets)	Purcell et al., 2014[27]	398	0.340	0.299	0.023	0.012	0.037	0.234	**7.07E-05**	0.211
microRNA-137 (TargetScan v6.2)	Ripke et al., 2013[26]	1064	0.133	0.387	0.071	0.099	0.030	0.189	0.133	0.349
microRNA-137 (TargetScan v5)	Lewis et al., 2005[24]	271	0.147	0.498	0.016	0.476	0.042	0.311	0.100	0.062
CACN (calcium channel voltage dependent) subunits	Ripke et al., 2013[26]	26	0.490	0.764	0.834	0.754	0.584	0.323	0.106	0.986
Synapse: Cell adhesion and trans synaptic signaling	Lips et al., 2012[19]	76	0.462	0.955	0.088	0.491	0.496	0.543	0.706	0.657
Synapse: Excitability	Lips et al., 2012[19]	57	0.338	0.988	0.939	0.709	0.479	0.396	0.777	0.987
Synapse: Intracellular signal transduction	Lips et al., 2012[19]	148	0.142	0.021	0.031	0.040	0.524	0.386	0.055	0.471
Synapse: Structural plasticity	Lips et al., 2012[19]	93	0.442	0.193	0.269	0.474	0.818	0.169	0.638	0.295
Oligodendrocytes: DNA metabolism: Gene transcription	Goudriaan et al., 2014[21]	123	0.449	0.770	0.480	0.355	0.540	0.663	0.583	0.545

Oligodendrocytes: Lipid metabolism	Goudriaan et al., 2014[21]	83	0.093	0.168	0.135	0.401	0.420	0.043	0.396	0.295
Oligodendrocytes: Oxidation reduction	Goudriaan et al., 2014[21]	50	0.880	0.180	0.392	0.671	0.571	0.291	0.088	0.371
Astrocytes: DNA metabolism: Transcription	Goudriaan et al., 2014[21]	113	0.359	0.276	0.232	0.056	0.353	0.958	0.119	0.748
Astrocytes: Signal transduction	Goudriaan et al., 2014[21]	346	0.227	0.245	0.182	0.226	0.529	0.124	0.598	0.695
Astrocytes: Signal transduction: G protein receptor	Goudriaan et al., 2014[21]	61	0.738	0.521	0.954	0.186	0.628	0.589	0.818	0.815
Astrocytes: Signal transduction: Tyrosine kinase	Goudriaan et al., 2014[21]	27	0.047	0.636	0.421	0.176	0.805	0.417	0.695	0.322
Astrocytes: Signal transduction: Small GTPase mediated	Goudriaan et al., 2014[21]	61	0.499	0.112	0.505	0.675	0.378	0.256	0.526	0.664
Astrocytes: Cell adhesion	Goudriaan et al., 2014[21]	81	0.092	0.810	0.300	0.023	0.877	0.666	0.544	0.888
Nuclear proteins	Kirov et al., 2012[23]	157	**3.50E-05**	0.766	0.701	0.712	0.972	0.163	0.237	0.171
Presynapse	Kirov et al., 2012[23]	403	0.404	0.641	0.735	0.903	0.529	0.712	0.918	0.908
Presynapse: Synaptic vesicle	Kirov et al., 2012[23]	323	0.551	0.475	0.748	0.811	0.713	0.834	0.851	0.912
Post Synaptic Density (all proteins)	Kirov et al., 2012[23]	650	0.081	0.017	0.214	0.402	0.083	0.437	0.096	0.385
Post Synaptic Density: ARC complex	Kirov et al., 2012[23]	25	0.474	0.851	0.814	0.235	0.369	0.124	0.142	0.465
Post Synaptic Density: NMDAR complex	Kirov et al., 2012[23]	59	0.447	0.858	0.792	0.645	0.100	0.043	0.119	0.129
Post Synaptic Density: 95 complex	Kirov et al., 2012[23]	56	0.931	0.872	0.952	0.586	0.208	0.089	0.346	0.464
Post Synaptic Density: mGluR5 complex	Kirov et al., 2012[23]	36	0.113	0.219	0.738	0.294	0.879	0.109	0.054	0.301
Mitochondrial proteins	Kirov et al., 2012[23]	188	0.969	0.289	0.326	0.614	0.124	0.294	0.374	0.361
MitoCarta	Pagliarini et al., 2008[25]	947	0.922	0.206	0.606	0.990	0.702	0.574	0.616	0.366
FMRP targets	Darnell et al., 2011[22]	760	0.018	0.978	0.130	0.099	0.154	0.080	0.086	0.212

* Uncorrected competitive *P* Values. In Bold: statistically significant *P* values after multiple testing correction for 264 tests.
ICV = Intracranial Brain Volume. GWAS = Genome Wide Association Analysis. CNV = Copy number variant. SNP = Single Nucleotide Polymorphism. mGluR5 = metabotropic glutamate receptor 5. NMDAR = N-methyl-D-spartate receptor. FMRP = Fragile X Mental Retardation Protein.

In a previous large GWAS this gene was found to be associated with the lentiform nucleus volume (this nucleus comprises the putamen and the globus pallidus within the basal ganglia), and deficits in lentiform nucleus volume have been implicated in a number of genetically influenced disorders, including Parkinson's disease, schizophrenia, and ADHD[28], suggesting that genetic mechanisms driving the development of the putamen are shared by several neuropsychiatric disorders.

In summary we found that two gene sets were previously implicated in schizophrenia are also associated with brain regions that may play a role in the pathophysiology of this complex psychiatric disorder. Our findings suggest that there is a shared genetic component that converges in gene sets associated with schizophrenia and volumetric brain phenotypes; understanding this link may provide a better insight of the genetic mechanisms driving brain development and this psychiatric disorder.

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6.8 SUPPLEMENTARY MATERIAL

Supplementary Table S1
Gene Based Association Analysis of the individual genes within the Nuclear Proteins Gene Set
(N Genes = 157)

GENE ID	CHROMOSOME	N SNPS	Z STAT	P
84188	11	105	2.8153	0.0024369
5019	5	170	2.5929	0.0047582
3992	11	25	2.3385	0.0096812
26354	3	15	2.3321	0.0098482
79709	19	64	2.2571	0.012
10658	11	77	2.245	0.012383
10130	2	163	2.2214	0.013163
7341	2	60	2.2081	0.013618
7431	10	11	2.0286	0.021252
682	19	28	2.0176	0.021818
23020	2	25	1.9279	0.026931
6605	17	17	1.8887	0.029468
7919	6	62	1.8555	0.031765
3190	9	12	1.8248	0.034018
6629	20	35	1.8231	0.034148
3015	4	6	1.8109	0.035078
3182	5	12	1.788	0.036884
27339	11	23	1.7502	0.040045
10961	12	11	1.745	0.040492
10291	22	74	1.6815	0.046332
476	1	39	1.5888	0.056053
2317	3	344	1.5709	0.058101
9221	10	11	1.5008	0.066708
7150	20	141	1.4863	0.0686
3008	6	1	1.4697	0.07082
9219	11	7	1.4443	0.074329
2052	1	123	1.3521	0.088176

1655	17	8	1.3396	0.090191
5832	10	168	1.3363	0.090729
10432	11	7	1.3262	0.09239
10801	17	591	1.2771	0.10078
4000	1	118	1.2754	0.10108
4809	22	42	1.2516	0.10536
3183	14	108	1.2381	0.10785
3856	12	145	1.1935	0.11634
6710	14	266	1.0808	0.1399
3024	6	4	1.0633	0.14383
10424	4	32	1.0606	0.14444
3066	6	57	1.0576	0.14512
6558	5	156	0.97794	0.16405
1595	7	34	0.97193	0.16554
5106	14	8	0.94959	0.17116
9188	10	55	0.94912	0.17128
338785	12	21	0.89887	0.18436
6635	1	34	0.88714	0.1875
10952	9	18	0.85699	0.19572
10949	5	2	0.85682	0.19577
488	12	131	0.85247	0.19698
23451	2	36	0.83262	0.20253
55505	15	10	0.81359	0.20794
871	11	28	0.80507	0.21039
55749	10	102	0.7966	0.21284
9782	5	39	0.77834	0.21818
8294	6	1	0.76714	0.2215
10521	22	25	0.76599	0.22184
3184	4	33	0.68431	0.24689
3007	6	3	0.68342	0.24717
3880	17	5	0.67983	0.2483
26097	1	23	0.60937	0.27114
1660	1	79	0.60367	0.27303
8106	14	10	0.54645	0.29238
51602	2	64	0.51796	0.30224
3005	22	3	0.51682	0.30264
51592	1	245	0.4769	0.31672
10212	19	12	0.46527	0.32087

10514	17	14	0.45036	0.32623
1785	19	228	0.38327	0.35076
400	12	26	0.38131	0.35149
3017	6	14	0.34673	0.3644
7879	3	222	0.32504	0.37258
6426	17	6	0.3127	0.37725
8539	11	54	0.30414	0.38051
4839	12	10	0.30273	0.38105
6626	19	31	0.30254	0.38112
220988	2	12	0.29793	0.38288
2521	16	4	0.29511	0.38396
10250	1	20	0.27057	0.39336
5898	7	162	0.26929	0.39385
10155	19	6	0.26497	0.39552
9868	3	83	0.2405	0.40497
4670	19	122	0.23926	0.40545
3875	12	4	0.2371	0.40629
54474	17	30	0.22369	0.4115
2091	19	15	0.19971	0.42086
79026	11	182	0.17071	0.43223
3852	12	26	0.15778	0.43732
23191	15	359	0.14213	0.44349
9555	5	85	0.12457	0.45043
121391	12	52	0.12092	0.45188
4173	8	6	0.11499	0.45422
1108	12	58	0.10698	0.4574
8470	4	1361	0.059437	0.4763
23560	10	76	0.058929	0.4765
382	14	1	0.040869	0.4837
10657	1	5	-0.027833	0.5111
10594	17	82	-0.10151	0.54043
3673	5	374	-0.12044	0.54793
23435	1	17	-0.13736	0.55463
7082	15	151	-0.13751	0.55469
3181	7	19	-0.1474	0.55859
94081	5	180	-0.15355	0.56102
3014	11	2	-0.19384	0.57685
6428	6	29	-0.19994	0.57923

3872	17	2	-0.23077	0.59125
7175	1	115	-0.23567	0.59316
51703	10	81	-0.23681	0.5936
6432	2	5	-0.24256	0.59583
93974	1	5	-0.25357	0.60009
26589	15	5	-0.28748	0.61313
5479	15	10	-0.29459	0.61585
6632	18	18	-0.3629	0.64166
10946	1	63	-0.37418	0.64587
7913	6	73	-0.38458	0.64972
6421	1	16	-0.38539	0.65003
3959	17	18	-0.3976	0.65454
4869	5	53	-0.40573	0.65753
23450	16	52	-0.4097	0.65899
22913	20	95	-0.43392	0.66783
6431	20	8	-0.44457	0.67169
10915	5	138	-0.45172	0.67426
54578	2	264	-0.50454	0.69306
55660	2	117	-0.5151	0.69676
4001	5	154	-0.53258	0.70284
348093	15	62	-0.5337	0.70323
23468	12	40	-0.54022	0.70548
1994	19	77	-0.5662	0.71437
3192	1	8	-0.58791	0.7217
1727	22	86	-0.62233	0.73314
27247	2	85	-0.64066	0.73913
5339	8	113	-0.6426	0.73976
8880	1	55	-0.71046	0.76129
6625	19	36	-0.73563	0.76902
3854	12	17	-0.73619	0.76919
51593	7	28	-0.75744	0.77561
11030	8	329	-0.75925	0.77615
55766	12	9	-0.76614	0.7782
29920	1	7	-0.76913	0.77909
26509	10	633	-0.77898	0.782
3849	12	14	-0.7866	0.78424
6734	11	18	-0.78661	0.78425
1737	11	48	-0.81898	0.7936

29957	1	190	-0.8554	0.80384
10528	20	14	-0.93912	0.82616
11338	19	38	-0.94248	0.82703
821	5	56	-0.94288	0.82713
84324	12	38	-0.94312	0.82719
800	7	454	-0.96313	0.83226
3855	12	42	-1.011	0.84399
6601	12	10	-1.1572	0.87641
3178	12	3	-1.2069	0.88626
9218	18	107	-1.2306	0.89077
3006	6	5	-1.2426	0.893
3009	6	5	-1.3043	0.90394
5899	2	110	-1.3934	0.91825
2130	22	46	-1.4402	0.92509
9775	17	32	-1.6144	0.94678
4926	11	106	-1.7943	0.96362

Supplementary Table S2
Gene Based Association Analysis of the individual genes within the High Confidence Targets of microRNA-137 Gene Set (N Genes = 398)

GENE ID	CHROMOSOME	N SNPS	Z STAT	P
9909	1	17	3.6941	0.0001103
4905	17	54	3.5345	0.0012043
114795	12	869	2.8878	0.0019395
9334	20	157	2.7913	0.0026248
1525	21	158	2.7178	0.003286
4440	12	45	2.7021	0.0034453
23286	5	540	2.5997	0.0046646
8707	1	11	2.5647	0.0051637
166614	4	462	2.5562	0.0052908
246175	4	149	2.5314	0.0056804
23067	12	29	2.5121	0.0060006
23131	17	119	2.4855	0.0064676
7068	3	995	2.4822	0.0065294
10106	12	30	2.451	0.0071232
57406	3	187	2.4434	0.0072738
10891	4	250	2.3891	0.0084451
161145	14	150	2.3156	0.010291
7707	3	230	2.2076	0.013636
79837	12	18	2.207	0.013658
10605	5	61	2.1742	0.014845
80205	16	501	2.1494	0.015799
4763	17	262	2.1095	0.017452
129642	2	182	2.0952	0.018077
8660	13	88	2.0663	0.019402
359	12	20	2.0191	0.021736
2288	12	5	2.012	0.022108
6158	19	16	1.9992	0.022791
63976	1	1099	1.9912	0.02323
144108	11	73	1.9714	0.024337
64478	8	12847	1.9517	0.025489
57154	7	142	1.8845	0.02975

1523	7	1029	1.8687	0.030832
4772	18	525	1.8421	0.032733
3131	17	117	1.8321	0.033466
5738	1	193	1.827	0.033849
253260	5	252	1.8225	0.034188
50999	1	49	1.8067	0.035408
2020	7	24	1.7904	0.036697
84867	11	137	1.7827	0.037314
150864	2	159	1.7818	0.037394
114907	8	121	1.7662	0.038685
5045	15	20	1.7043	0.044161
64756	1	58	1.703	0.044286
23762	22	523	1.6898	0.045533
113878	7	17	1.6355	0.05097
9912	17	678	1.6033	0.054439
51108	16	43	1.5742	0.057717
160897	13	54	1.5492	0.060662
11057	15	195	1.5118	0.065296
64137	11	18	1.4896	0.068161
50717	1	44	1.4885	0.068313
399947	11	3	1.4674	0.071134
150094	21	45	1.4497	0.07357
84890	10	6	1.4396	0.074996
84279	2	10	1.4325	0.075998
130340	2	287	1.4324	0.076022
2932	3	368	1.4322	0.076045
57538	15	137	1.3685	0.085584
162427	17	34	1.3647	0.086169
9701	22	270	1.3566	0.087461
7629	6	79	1.3413	0.089912
388228	16	21	1.2795	0.10036
10765	1	174	1.2741	0.10131
57493	3	234	1.2722	0.10165
65264	17	48	1.2577	0.10424
256364	11	17	1.2528	0.10513
6558	5	146	1.2436	0.10682
133522	5	366	1.241	0.1073
2977	11	927	1.2326	0.10886

23167	8	309	1.2275	0.10982
22843	17	291	1.2097	0.1132
9175	3	414	1.2073	0.11366
375056	1	96	1.2006	0.11495
5562	5	65	1.1975	0.11556
11278	13	1206	1.169	0.12121
22998	4	684	1.166	0.12181
58528	6	95	1.1608	0.12286
27254	22	25	1.1285	0.12956
2779	3	4	1.1078	0.13397
23253	18	358	1.1077	0.13399
55225	1	131	1.0998	0.13571
10559	6	102	1.096	0.13655
3815	4	162	1.0942	0.13693
2104	1	1841	1.0938	0.13703
65983	5	343	1.0934	0.13712
7326	17	104	1.0866	0.13861
4747	8	8	1.0631	0.14387
6596	3	102	1.0576	0.14511
148534	1	217	1.0574	0.14517
64101	7	5	1.0359	0.15012
23659	16	24	1.0186	0.1542
22863	14	146	1.0137	0.15537
57713	10	772	1.0062	0.15716
10435	11	7	0.98503	0.16231
8911	22	242	0.9839	0.16258
4851	9	113	0.97817	0.16399
1995	19	57	0.97611	0.1645
79953	20	452	0.9673	0.1667
23331	22	1068	0.96709	0.16675
284353	19	15	0.96174	0.16809
83482	8	2	0.95724	0.16922
286336	9	20	0.94981	0.1711
22853	7	215	0.94892	0.17133
64328	13	231	0.9448	0.17238
57494	12	43	0.9315	0.1758
55536	7	138	0.87775	0.19004
340348	7	53	0.87546	0.19066

8303	16	29	0.86946	0.1923
54557	5	81	0.8465	0.19864
1113	14	38	0.83196	0.20272
252995	1	9	0.82984	0.20331
23151	22	168	0.82835	0.20373
11060	16	356	0.81931	0.2063
63941	20	10	0.81567	0.20734
23244	4	334	0.81312	0.20808
6138	3	10	0.80747	0.2097
83992	7	300	0.80554	0.21026
2101	11	8	0.80184	0.21132
7020	6	29	0.78077	0.21747
4665	12	14	0.77943	0.21786
6854	3	102	0.77025	0.22057
9895	14	378	0.76279	0.2228
6913	1	256	0.76097	0.22334
143872	11	1135	0.75581	0.22488
3224	12	2	0.75369	0.22552
6239	6	251	0.74999	0.22663
57455	19	59	0.745	0.22813
4286	3	457	0.71183	0.23828
2849	10	83	0.71165	0.23834
25946	12	30	0.70114	0.24161
56957	1	49	0.68461	0.24679
84662	16	8	0.67034	0.25132
8452	2	257	0.66187	0.25403
5829	12	51	0.65032	0.25774
22889	1	19	0.64575	0.25922
3756	1	1310	0.63665	0.26218
766	16	13	0.61929	0.26786
27348	9	17	0.58254	0.2801
55222	10	272	0.57893	0.28132
1289	9	785	0.5721	0.28363
8854	15	305	0.5675	0.28519
7222	4	111	0.55505	0.28943
5564	12	17	0.52305	0.30047
79001	16	5	0.51397	0.30364
7782	15	28	0.49728	0.3095

25778	1	144	0.49612	0.30991
55917	1	132	0.49389	0.31069
5210	3	32	0.48875	0.31251
55279	3	9	0.47125	0.31873
342371	16	10	0.46933	0.31942
10846	6	1015	0.4652	0.32089
92140	8	139	0.46363	0.32146
10184	5	290	0.46335	0.32156
23143	13	456	0.45568	0.32431
163486	1	266	0.4538	0.32499
7003	11	391	0.45328	0.32517
55188	12	169	0.43949	0.33015
4864	18	76	0.43283	0.33257
23648	1	393	0.42288	0.33619
2045	6	386	0.41886	0.33766
54149	21	36	0.41601	0.3387
1310	6	710	0.39493	0.34645
57646	11	136	0.38679	0.34945
7257	1	44	0.38087	0.35165
100113407	6	132	0.37859	0.3525
23216	4	725	0.36344	0.35814
285671	5	286	0.32443	0.37281
254048	7	89	0.31937	0.37472
117178	1	105	0.31692	0.37565
23180	3	568	0.31326	0.37704
6095	15	1757	0.30371	0.38067
9261	1	105	0.29631	0.3835
6487	1	427	0.29098	0.38553
23607	6	273	0.28208	0.38894
390205	11	6	0.25196	0.40054
220164	18	1459	0.23411	0.40745
283373	12	13	0.23358	0.40766
10645	12	141	0.22949	0.40924
140901	20	105	0.225	0.41099
4781	9	566	0.20889	0.41727
51334	5	743	0.20712	0.41796
96459	5	181	0.2067	0.41812
5577	7	225	0.20127	0.42024

83698	7	1525	0.19428	0.42298
81606	2	77	0.192	0.42387
8867	21	115	0.18926	0.42494
3760	2	416	0.18894	0.42507
83931	1	42	0.18857	0.42521
444	8	453	0.17308	0.43129
1832	6	113	0.15577	0.43811
81832	18	420	0.15048	0.44019
65059	2	118	0.14692	0.4416
6497	1	175	0.14674	0.44167
8476	1	1440	0.14635	0.44182
9771	7	658	0.14243	0.44337
85379	22	402	0.12008	0.45221
1112	14	1099	0.1128	0.45509
56848	19	10	0.10944	0.45643
56894	21	271	0.10393	0.45861
11156	8	16	0.083029	0.46691
5432	16	19	0.082123	0.46727
64795	2	52	0.073934	0.47053
57468	20	115	0.056502	0.47747
8648	2	307	0.049421	0.48029
1846	8	43	0.033896	0.48648
5306	17	111	0.030076	0.488
126567	19	7	0.027239	0.48913
9568	9	1418	0.0068125	0.49728
23370	19	301	0.0019244	0.49923
56995	6	577	0.00040397	0.49984
11044	5	173	-0.00049051	0.5002
775	12	1436	-0.008046	0.50321
23386	7	167	-0.014617	0.50583
9586	7	1453	-0.016384	0.50654
54788	10	48	-0.018874	0.50753
6815	14	71	-0.031411	0.51253
9794	5	86	-0.032556	0.51299
64599	7	16	-0.047684	0.51902
678	2	7	-0.066431	0.52648
6047	4	97	-0.069486	0.5277
9318	15	70	-0.070631	0.52815

145501	14	67	-0.073646	0.52935
64375	12	12	-0.073715	0.52938
7750	13	190	-0.078689	0.53136
81542	14	42	-0.083522	0.53328
10015	3	204	-0.097517	0.53884
115207	13	11	-0.098727	0.53932
5500	2	100	-0.098844	0.53937
3613	18	228	-0.10032	0.53995
394	14	40	-0.10805	0.54302
317762	14	303	-0.12818	0.551
8202	20	282	-0.1295	0.55152
6431	20	7	-0.14193	0.55643
60482	2	70	-0.15288	0.56075
9444	6	291	-0.15672	0.56227
10818	12	238	-0.17311	0.56872
54861	3	62	-0.17475	0.56936
27230	3	9	-0.17882	0.57096
9682	1	70	-0.17982	0.57135
23232	10	198	-0.18074	0.57172
10018	2	87	-0.18178	0.57212
23114	1	492	-0.18634	0.57391
58158	12	18	-0.18787	0.57451
9628	14	2009	-0.20325	0.58053
143684	11	52	-0.22394	0.5886
1847	10	19	-0.22736	0.58993
55206	12	161	-0.23518	0.59296
53358	9	448	-0.23744	0.59384
6767	22	28	-0.23799	0.59405
10242	3	824	-0.23806	0.59408
91746	4	34	-0.23839	0.59421
166968	5	82	-0.2408	0.59515
3416	10	232	-0.24408	0.59642
92181	5	197	-0.25622	0.60111
3101	5	39	-0.25687	0.60136
57605	12	67	-0.27568	0.6086
56937	20	167	-0.27669	0.60899
154467	6	33	-0.28229	0.61114
90102	3	800	-0.28536	0.61232

6536	1	58	-0.28579	0.61248
6801	2	283	-0.31093	0.62207
283464	12	119	-0.32184	0.62621
140730	20	84	-0.3221	0.62631
23508	14	80	-0.32237	0.62641
151354	2	4	-0.33534	0.63131
93664	7	1167	-0.33894	0.63267
114794	22	189	-0.34365	0.63445
55156	8	109	-0.35305	0.63798
9697	6	287	-0.35613	0.63913
5141	19	73	-0.35897	0.64019
6416	17	138	-0.36148	0.64113
5871	11	6	-0.38232	0.64889
5602	4	1079	-0.41046	0.65926
9542	5	352	-0.4108	0.65939
120114	11	929	-0.41146	0.65963
84620	2	274	-0.41181	0.65976
25769	9	657	-0.41751	0.66185
5784	1	659	-0.41799	0.66202
113829	5	6	-0.42337	0.66399
3192	1	8	-0.43389	0.66782
54464	3	157	-0.44677	0.67248
202915	7	42	-0.45468	0.67533
220988	2	12	-0.45521	0.67552
374655	15	132	-0.45713	0.67621
4094	16	12	-0.46122	0.67768
55964	22	46	-0.46809	0.68014
11217	9	337	-0.47182	0.68147
23274	16	552	-0.47744	0.68347
80301	15	58	-0.49789	0.69072
23122	3	341	-0.50385	0.69282
4761	17	4	-0.51257	0.69587
203190	8	14	-0.51289	0.69599
23452	9	46	-0.5325	0.70281
3417	2	24	-0.53837	0.70484
84749	12	42	-0.54006	0.70542
8912	16	121	-0.55281	0.7098
23609	3	58	-0.56459	0.71382

23243	3	327	-0.57728	0.71813
1399	22	80	-0.57857	0.71856
84945	13	40	-0.58889	0.72203
55284	8	98	-0.59136	0.72286
10672	17	14	-0.6043	0.72718
55161	4	56	-0.60579	0.72767
23144	8	253	-0.61338	0.73019
11329	6	131	-0.61851	0.73188
6714	20	95	-0.62026	0.73246
8927	3	122	-0.62047	0.73253
253827	12	250	-0.62877	0.73525
51128	5	25	-0.63229	0.7364
4801	12	38	-0.63655	0.73779
9497	3	317	-0.64369	0.74011
9706	17	114	-0.64834	0.74162
729993	16	1113	-0.64933	0.74194
23037	5	957	-0.64977	0.74208
4520	1	49	-0.65782	0.74467
50862	11	78	-0.66957	0.74843
147339	18	115	-0.67175	0.74913
338645	11	2100	-0.67263	0.74941
9806	10	78	-0.67812	0.75115
107	7	361	-0.68393	0.75299
8462	2	26	-0.69498	0.75647
51059	8	1060	-0.70633	0.76001
2554	5	117	-0.72088	0.76451
51341	19	25	-0.74844	0.7729
79668	5	258	-0.75635	0.77528
285172	2	148	-0.75694	0.77546
90113	3	21	-0.75993	0.77635
6925	18	619	-0.76259	0.77715
808	19	15	-0.77055	0.77951
3736	12	14	-0.77219	0.78
57484	4	786	-0.77756	0.78159
23112	22	391	-0.78072	0.78252
283248	11	4	-0.78983	0.78519
108	5	1046	-0.79465	0.78659
56975	7	79	-0.79856	0.78773

11343	3	306	-0.81667	0.79294
3090	17	11	-0.82163	0.79436
23382	7	341	-0.82534	0.79541
8916	4	209	-0.84037	0.79965
25836	5	211	-0.85003	0.80234
8139	16	274	-0.85341	0.80328
116159	21	256	-0.85546	0.80385
8503	1	142	-0.85968	0.80502
9515	3	1339	-0.86207	0.80567
3632	10	589	-0.88271	0.8113
7082	15	149	-0.90308	0.81676
4084	2	40	-0.90371	0.81692
26035	15	134	-0.90578	0.81747
55041	2	24	-0.91143	0.81897
6546	2	1231	-0.92363	0.82216
6529	3	140	-0.96919	0.83378
85414	1	55	-1.0253	0.84739
26051	20	262	-1.0378	0.85031
901	4	23	-1.0438	0.85171
51043	1	20	-1.0505	0.85326
2802	12	189	-1.0665	0.85691
6387	10	55	-1.0909	0.86234
153	10	2	-1.1124	0.86703
55607	7	613	-1.1168	0.86797
8879	10	174	-1.1548	0.87592
124935	17	104	-1.1571	0.87638
2589	18	131	-1.1594	0.87685
51304	3	81	-1.1714	0.87929
57719	19	23	-1.1825	0.8815
6263	15	1980	-1.1846	0.88192
815	5	123	-1.1853	0.88204
4642	17	608	-1.2041	0.88571
28996	7	84	-1.2147	0.88777
11228	12	421	-1.2442	0.89328
91526	2	774	-1.2492	0.8942
2046	1	138	-1.2513	0.8946
56261	20	148	-1.2603	0.89622
6926	12	16	-1.2781	0.8994

79805	1	98	-1.2867	0.90091
57568	1	326	-1.3101	0.90492
340481	9	294	-1.3134	0.90548
6445	13	887	-1.3416	0.91014
84883	10	48	-1.3625	0.91348
138151	9	238	-1.3874	0.91734
8504	6	54	-1.3971	0.91881
3021	17	9	-1.4278	0.92333
26003	2	71	-1.445	0.92577
2256	17	7	-1.4466	0.926
8932	18	108	-1.4748	0.92987
53373	12	98	-1.5123	0.93477
9765	5	146	-1.519	0.93561
23250	13	433	-1.5218	0.93597
115294	8	248	-1.5579	0.94037
91404	2	321	-1.585	0.94351
339983	4	8	-1.6417	0.94967
208	19	80	-1.6522	0.95076
85508	20	22	-1.7189	0.95718
51473	6	711	-1.7666	0.96135
2275	1	5	-1.7969	0.96383
610	19	16	-1.8348	0.96673
63027	6	632	-1.9133	0.97214
121512	12	462	-2.0126	0.97792
26030	14	67	-2.0794	0.98121

Chapter 7

Summary and Future Directions

CHAPTER 7

SUMMARY AND FUTURE DIRECTIONS

Imaging genetics is a research tool that uses neuroimaging and genetics to assess the impact of genetic variation on brain function and structure. Imaging genetics can be used to identify genes for complex traits such as cognition; as well to characterize the neural systems affected by risk genetic variants to elucidate quantitative and mechanistic aspects of brain function implicated in complex neuropsychiatric disorders[1]. At the start of my PhD project in 2010, the field of genetics of complex traits was dealing with the so called "missing heritability" problem[2], at that time genome-wide association studies had provided valuable insights into the genetic basis of human disease; but they had explained relatively little of the heritability of most complex brain traits, and the variants identified through those studies had small effect sizes.

Many groups had started looking for alternative methods to overcome the "missing heritability" problem; one of those methods was imaging genetics, as many groups were starting to apply genetics to neuroimaging techniques to study complex brain traits. Genetic studies of functional and structural neuroimaging phenotypes were performed under the assumption that those brain measures were "intermediate phenotypes", under this hypothesis, the brain imaging phenotypes were postulated to be closer to the biological pathway of genes than the behavioral phenotype itself[3].

The first imaging genetic studies primarily focused on studying association between brain morphology and single nucleotide polymorphisms of genes of known function, such as catechol-O-methyltransferase (*COMT*)[4], brain-derived neurotrophic factor (*BDNF*)[5], and disrupted-in-schizophrenia 1 (*DISC1*)[6], among other genes. Many GWAS studies of complex brain traits had identified a handful of genes associated with psychiatric disorders with unknown functions; for example the *ZNF804A* gene was associated with schizophrenia[7], and imaging genetics was used to investigate the biological function of this gene[8-10]. Many of these findings were limited by very small number of studies reported for each gene/SNP, the small sample sizes, and differences in the methodology of brain morphological measurements, and in the tested anatomical regions[1].

7.1 SUMMARY

In this thesis I presented 4 different original research studies looking at the genetics of complex brain traits using novel statistical methods (i.e. gene set analyses, and polygenic analysis) to analyze large data sets obtained from genetics and neuroimaging studies.

In chapter 3, I presented a study looking at the effect in the brain morphology of a set of genes previously associated with cognitive ability[11] using a novel VBM analysis for genetic studies. In this study we performed an association analysis using a mass univariate model exploring the effect of 502 SNPs within 25 G-protein genes on gray matter volume; this was a novel approach in the imaging genetic field as most VBMs studies only looked a the effect of one genetic variant at the time. This study was performed in a relatively large sample for most imaging studies standards at the time (532 healthy individuals). In order to avoid the well-known interscanner confounding effect, the sample set was divided into 2 groups according to the MRI scanner used; 294 subjects were scanned in a 3-T scanner, and 238 subjects were scanned in a 1.5-T scanner.

To increase the power to detect association, the larger sample was used as a discovery sample and the smaller sample was used as an independent sample for replication. We found that SNPs in 7 G-proteins genes (*GNG2, GNAQ, GNA14, GNA15, GNAO1, GNAL, GNB5*) were significantly associated with differences in 3 brain regions: the medial frontal cortex, the temporal lobe, and the occipital lobe. Interestingly, variants in 4 of the significantly associated G-proteins genes (*GNG2, GNAQ, GNA15, GNA14*) were associated with gray matter changes in an overlapping region of the medial frontal cortex. We conducted a second study to verify our initial result in an independent sample and were able to replicate the association for 2 of the 4 genes (*GNG2* and *GNAQ* genes) in the exact same brain region of the medial frontal cortex as the discovery sample.

The complexity of the high-volume data generated in this VBM imaging genetics study was prone to produce too many false positives or false negatives. Even when our approach allowed us to prevent high rate of false positive findings, this approach might also increase the risk of type II errors, which at the end might represent

the major limitation of this study. The multiple layers of statistical protection and the independent replication analysis strengthen the conviction that the observed associations of gray matter volume variation in the medial frontal cortex with markers in *GNG2* and *GNAQ* genes are genuine.

In chapters 4, 5, and 6; I focused on studying the imaging genetics of schizophrenia; a complex neuropsychiatric disorder that is clinically characterized by positive and negative psychotic symptoms, as well as by diverse cognitive deficits. I chose to study this phenotype because as a psychiatrist I'm very interested in this neurodevelopmental disorder that affects approximately 1% of the population worldwide[12], and which has an enormous impact at personal, societal, medical and economic levels[13]. Schizophrenia is a highly heritable disorder[14], with a complex genetic etiology; although there are a number of robust genetic variants that have recently been identified as risk factors[15-17], the underlying neurobiology of this chronic disorder remains elusive.

During the last decade several research groups around the world have collaborated to create The Psychiatric Genomics Consortium (PGC), this consortium was established to promote rapid progress towards the identification of genetic causes underlying several psychiatric disorders, including schizophrenia. The advantage of this consortium is that the outcomes of all the genetic studies are publicly available, and it was very useful for the completion of this PhD project.
In chapter 4, and 5 I presented two studies looking at the effect that a different genes have on the white matter integrity of the brain in the subjects with schizophrenia and healthy controls. This work was done in collaboration The MCIC Collection (A Shared Repository of Multi-Modal, Multi-Site Brain Image Data from a Clinical Investigation of Schizophrenia)[18].

White matter integrity (as measured by FA) was chosen as the phenotype of interest for studies presented in these two chapters because it provides a good plausible biological endophenotype to explain genetic differences in the risk for schizophrenia[19,20]. Disruptions in white matter tract structures have been consistently implicated in the pathophysiology of schizophrenia; a large number of diffusion tensor imaging studies in schizophrenia have found white matter abnormalities in various brain regions[21-25]. Of all the diffusion tensor imaging parameters, FA of water diffusion has the highest reported heritability[26].

It is unclear how white matter microstructural abnormalities relate to the underlying genetic architecture of Schizophrenia, nevertheless the endophenotypic importance of FA for schizophrenia is further supported by recent studies showing that many brain regions have significant decrease in FA in subjects with schizophrenia (including childhood-onset schizophrenia), and were also decreased similarly but with smaller effects in their unaffected relatives; with a continuous FA decrease from healthy subjects to relatives to subjects with schizophrenia[27, 28].

Although there is a lack of consistency in the spatial localization of the brain regions showing reduced FA, in this regard it has been found that white matter abnormalities are not localized to a specific brain region but instead reflect a diffuse process with widely dispersed focal reductions in FA that vary spatially among individuals[29-31].

In chapter 4, I presented a gene set association analysis using a modified version of the oligodendrocyte gene sets derived from expert-curated glial gene sets[32]. The gene set association analysis was performed using a novel statistical software package developed by our group: the Joint Association of Genetic Variants (JAG) software (http://ctglab.nl/software/). This software allows testing the combined effect of all genetic variants available within a gene set[33]. We found a statistically significant association between schizophrenia and FA, between the myelination gene set and schizophrenia, and between the myelination gene set and FA. Additionally, we tested for the association between the myelination gene set and schizophrenia; while correcting for FA, this association remains significant, indicating that the variance in schizophrenia liability is partially explained by the myelin gene set independently of the variance explained by FA.

On the other hand, when testing the association between the myelination gene set and FA while correcting for schizophrenia status, this association was no longer statistically significant; this suggests that the variance observed for total brain FA is explained only in part by the myelination gene set, and that there are other genetic variants associated with schizophrenia that have an effect on FA and are not included in the myelination gene set. As in many other imaging genetic studies, this study was limited by sample size.

The gene set analysis has the limitation that requires prior knowledge of the biological function of known genes, in other words, this approach is similar to the candidate gene analysis, but instead of looking at a single genetic variant, it looks at several genetic variants of genes within a plausible biological functional group.

In order to overcome this limitation; in chapter 5, I presented a study looking at the effect of a polygenic risk score for schizophrenia in the white matter integrity of the brain of subjects with schizophrenia and healthy controls. The polygenic risk scores are calculated from unbiased-selected set of genes from GWAS findings. Recently, the PGC reported a multi-stage schizophrenia GWAS of up to 36,989 cases and 113,075 controls, they identify 128 independent associations spanning 108 conservatively defined loci that meet genome-wide significance[34], the associations were enriched among genes expressed in brain, providing biological plausibility for the findings. We used the results from this large GWAS to calculate polygenic risk scores for schizophrenia in an independent sample provided by the MCIC collection to investigate whether those risk scores would explain a significant proportion of the variance of brain FA.

We first confirmed that the polygenic risk scores for schizophrenia predicted the case/control status in our independent sample, indicating that the risk profile score is a consistent measure of schizophrenia risk. Then we showed that FA was associated with schizophrenia case-control status, and that the polygenic risk scores for schizophrenia were progressively correlated with FA of the total brain in the complete target sample, and separately in both cases and controls. Our findings are specific to white matter integrity only, as none of the polygenic scores were found to be associated with other brain phenotypes, such as total brain volume, white matter volume, gray matter volume, or lateral ventricles volume.

From this study we can conclude that polygenic risk scores for schizophrenia can explain part of the inter-individual variation in white matter integrity, in both schizophrenia subjects and healthy controls. This finding is not limited to one specific brain region, suggesting a diffuse effect of schizophrenia-associated genetic variants on white matter integrity.

Finally, in chapter 6, I presented a gene set analysis using data publicly available thorough the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium; we obtained directly from ENIGMA the p-values of a GWAS conducted as part of a collaborative large-scale genetic analysis of magnetic resonance imaging scans to identify genetic variants that influence brain structure in over 13,000 healthy subjects. We conducted this gene-set analysis using MAGMA (Multi-marker Analysis of Genomic Annotation)[35], a novel and freely available software for gene and gene-set analysis of GWAS genotype data developed by our group (http://ctglab.nl/software/magma). MAGMA analysis can be performed from raw GWAS data, or from SNP p-values. For this study we used the p-values from the GWAS of different subcortical brain volumes (nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus), and ICV obtained from the ENIGMA consortium[36]. In the analysis we included expert-curated sets of genes that have shown to be significantly associated with schizophrenia based on whole genome approaches.

We found that at least two gene sets previously associated with schizophrenia (microRNA-137 target genes, and genes encoding for nuclear proteins) are also associated with brain regions that may play a role in the pathophysiology of this complex psychiatric disorder. Our findings suggest that there is a shared genetic component between gene sets associated with schizophrenia and volumetric brain phenotypes; understanding this link may provide a better insight of the genetic mechanisms driving brain development and this psychiatric disorder.

7.2 FUTURE DIRECTIONS

The constant development of novel and more complex genetic and neuroimaging approaches has introduced the field of imaging genetics to a new era. In this PhD project I applied several novel statistical approaches to analyze the genetics of complex brain traits.

In summary, the work in this study identified a gene set associated with cognitive ability that explained differences in grey matter volume of the medial prefrontal cortex; it also identified a link between myelination genes and white matter integrity,

as well as an association of polygenic risk scores of schizophrenia with white matter integrity. Finally, this work also elucidated a link between microRNA-137 target genes (a well-known genetic variant associated with schizophrenia) and the putamen.

This PhD thesis puts in evidence the complexity of the field of imaging genetics, and shows that analyzing large amounts of imaging and genetic data face a critical issue when it comes to correcting for multiple testing; and not to mention the computing power that is needed to run these kind of analyses. For example, performing a VBM study of hundreds or thousands of genetic findings from a GWAS analysis will require to perform separated statistical association analyses of hundreds of thousands of voxels, this kind of study involves millions of statistical tests that will require correction for multiple testing.

In order to correct for multiple comparisons in neuroimaging analyses, future studies will require defining a critical statistical threshold, especially if we take in consideration that a lot of these tests are not necessarily independent. In this project I tried to reduce the amount of data analyzed by grouping genes into sets with a plausible biological function; I also tried to limit the imaging phenotypes to those that have been previously associated with an specific disorder. Nonetheless, I also used very stringent statistical correction methods to ensure that our findings are not just due to chance; but by doing so, the power to detect true signals with small effect sizes was significantly reduced.

In future imaging genetics studies, the association analysis of large amounts of data will not only require novel methods for data reduction, but it will also have to increase the power to detect signals with small effect size. In order to increase statistical power, future studies will require to collect larger sample sizes, this might be achieved by collaborative efforts, such as the one currently being done by Psychiatric Genomics Consortium and the ENIGMA Consortium.

Future advances in imaging genetics will contribute to elucidate the neuronal basis of complex brain traits, such as cognition; as well as the physiopathology of complex neuropsychiatric disorders such as schizophrenia, bipolar disorder, and autism, among other brain disorders.

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Resumen en Español

(Spanish Summary)

SPANISH SUMMARY

RESUMEN

La genética de imágenes es una herramienta de investigación que utiliza neuroimágenes (generalmente imágenes de resonancia magnética) y la genética para evaluar el impacto que variantes genéticas pueden tener en la función y estructura del cerebro. La estudios de genética de imágenes se pueden utilizar para identificar genes associados con rasgos de comportamiento complejos (tales como la cognición); así como para caracterizar los sistemas neurales afectados por variantes genéticas de riesgo. Estos estudios tienen como fin dilucidar los posibles efectos que estos genes pueden tener en la función cerebral, y como estos pueden estar implicados en diferentes trastornos neuropsiquiátricos complejos.

En el 2010, al inicio de mi proyecto de tesis doctoral, el campo de la genética de los rasgos complejos estaba enfrentada con el llamado problema de la "heredabilidad faltante", en ese momento los estudios de asociación de genoma completo (GWAS) habían proporcionado información valiosa sobre la base genética de las enfermedades humanas; pero habían explicado relativamente poco la alta heredabilidad de los rasgos cerebrales más complejos. Las variantes genéticas de riesgo identificadas a través de esos estudios explicaban muy poco el problema estudiado, es decir el efecto estadístico explicado por estas variantes genéticas era de tamaño pequeño.

En ese entonces, muchos grupos de investigación habían empezado a buscar métodos alternativos para superar el problema de la "heredabilidad faltante"; uno de esos métodos fue el de genética de imágenes, muchos grupos habían empezando a combinar estudios genéticos con las técnicas de neuroimágen para estudiar rasgos complejos cognitivos y del comportamiento, así como enfermedades neuropsiquiátricas complejas. Se realizaron estudios genéticos de fenotipos cuantitativos de neuroimágen funcional y estructural, bajo el supuesto de que esas medidas eran "fenotipos intermedios", es decir, se postuló que los fenotipos de imágenes cerebrales estaban más cerca de los genes que el propio fenotipo conductual, por lo tanto era mas fácil encontrar los genes que explican la estructura o función cerebral, que encontrar genes que explicaran comportamientos complejos.

Los primeros estudios de genética de imágenes se centraron principalmente en estudios de asociación entre la morfología del cerebro y polimorfismos de nucleótidos únicos (SNP) de genes con función conocida, tales como el gen que codifica para la enzima catecol-O-metiltransferasa (COMT), el factor neurotrófico derivado del cerebro (BDNF), y el gen "interrumpido-en-esquizofrenia-1" (DISC1), entre otros genes. Al mismo tiempo, muchos estudios GWAS habían empezado a identificar genes con funciones desconocidas que estaban asociados con trastornos psiquiátricos; por ejemplo el gen ZNF804A se asoció con la esquizofrenia, y la genética de imágenes se utilizó para investigar la función biológica de este gen en el cerebro.

Muchos de estos hallazgos estaban limitados por el número pequeño de estudios reportados para cada gen/SNP, por el tamaño de las muestras de estudio (generalmente muy pequeñas), y las diferencias en la metodología usada para adquirir las mediciones morfológicas cerebrales, o en las regiones anatómicas cerebrales analizadas.

El constante desarrollo de nuevos y más complejos enfoques genéticos y de neuroimagen ha introducido el campo de la genética de imágenes en una nueva era. Este proyecto de tesis doctoral busca aprovechar varios de estos enfoques novedosos, especialmente en el area de la estadística genética, para explicar diferencias en algunos rasgos cerebrales complejos; como lo son la cognición y transtornos neuropsiquiatricos complejos.

En esta tesis se logró identificar que un conjunto de genes previamente asociados con la capacidad cognitiva también explican diferencias en el volumen de materia gris de la corteza prefrontal medial del cerebro. También se determinó una relación entre genes involucrados en el proceso de mielinización y la integridad estructural de la materia blanca del cerebro; así como una asociación del riesgo poligénico de la esquizofrenia con la integridad estructural de la materia blanca cerebral. Finalmente, este trabajo también dilucidó un vínculo entre genes diana del microARN-137 (una variante genética conocida asociada con la esquizofrenia) y el volumen del putamen en el cerebro.

Esta tesis doctoral pone en evidencia la complejidad del campo de la genética de imágenes, y muestra que el análisis de grandes cantidades de imágenes y datos genéticos se enfrentan a un problema crítico cuando se trata de corregir las múltiples

pruebas estadísticas; por no hablar del gran poder computacional que se requiere para ejecutar este tipo de análisis.

Con el fin de corregir el problema de comparaciones estadísticas múltiples en estudios genéticos de neuroimágen; los estudios de genética de imágenes futuros requieren la definición de un umbral estadístico crítico para determinar cuando un hallazgo es estadísticamente significativo, sobre todo si tomamos en cuenta que muchas de estas pruebas no son necesariamente independientes.

En este proyecto de tesis doctoral he tratado de reducir la cantidad de datos analizados mediante la agrupación de genes en grupos con una función biológica verosímil; también traté de limitar los fenotipos de imágenes a las que han sido previamente asociadas con un trastorno específico. No obstante, también utilicé métodos de corrección estadística muy estrictos para asegurar que nuestros hallazgos no se debieran simplemente a la casualidad; pero al hacerlo, se redujo significativamente la potencia para detectar señales con efecto estadístico pequeño.

En futuros estudios de genética de imágenes, el análisis de asociación de grandes cantidades de datos no sólo requerirá nuevos métodos para la reducción de la complejidad de los datos a analizar; sino que también tendrá que aumentar el poder estadístico para detectar señales con efecto pequeño. Con el fin de aumentar la potencia estadística, los estudios futuros requerirán colectar muestras de mayor tamaño, esto podría lograrse solo mediante esfuerzos de colaboración de diferentes grupos de investigación a nivel mundial, como los que actualmente están siendo realizados por Consorcio de Psiquiátrica Genómica y el Consorcio de Neuroimágenes ENIGMA.

Los futuros avances en la genética de imágenes contribuirán a dilucidar las bases neuronales de rasgos de comportamientos complejos, tales como la cognición; así como la fisiopatología de los trastornos neuropsiquiátricos complejos, tales como esquizofrenia, el trastorno bipolar, y el autismo; entre otros.

Nederlandse Samenvatting

(Dutch Summary)

DUTCH SUMMARY

SAMENVATTING

‘Imaging genetics’ is het onderzoeksveld dat een combinatie van neuroimaging (meestal magnetische resonantie beeldvorming) en genetica gebruikt om het effect van genetische variatie op hersenfunctie en structuur te onderzoeken. ‘Imaging genetics’ kan worden gebruikt voor de identificatie van genen voor complexe eigenschappen waarin het brein een belangrijke rol speelt, zoals cognitie en neuropsychiatrische aandoeningen.

Aan het begin van mijn PhD-project in 2010, had het gebied van de genetica van complexe eigenschappen te maken met het zogenaamde “ontbrekende erfelijkheid probleem”. Genoom breed onderzoek had weliswaar waardevolle inzichten in de genetische basis van ziekten bij de mens opgeleverd maar verklaarde slechts relatief weinig van de erfelijkheid van deze complexe eigenschappen. Dit omdat genetische varianten die werden geïdentificeerd elk slechts een klein effect op de ziekte hadden. Een deel van de erfelijkheid bleef daarmee ‘ontbreken’.

Om het probleem van de ”ontbrekende genen” op te lossen gingen verschillende onderzoeksgroepen op zoek naar alternatieve methoden. Een van deze methoden was ‘imaging genetics’ waarbij genetica werd gelinkt aan neuroimaging technieken waarmee complexe eigenschappen van de hersenen in kaart worden gebracht. De hypothese hierbij was dat functionele en structurele breineigenschappen als “intermediaire fenotypen” konden dienen. In onze zoektocht naar het biologische pad van gedrag naar de betrokken genetische factoren bevinden de hersenen zich immers dichterbij de genen dan het complexe gedrag zelf (bijvoorbeeld een psychiatrische stoornis).

De eerste ‘Imaging genetics’ studies richtten zich primair op het bestuderen van de associatie tussen hersenmorfologie en één genetische variant van genen waarvan de functie bekend was, zoals catechol-O-methyltransferase (COMT), hersenen-afgeleide neurotrophic factor (BDNF) en verstoord-in-schizofrenie 1 (DISC1).

GWAS-studies identificeerden tevens een handvol genen die geassocieerd waren met psychiatrische stoornissen maar waarvan de functie onbekend was. Het ZNF804A-gen werd bijvoorbeeld in verband gebracht met schizofrenie. Met behulp van 'imaging genetics' probeerde men de biologische functie van dit gen te onderzoeken. De resultaten van deze studies waren echter beperkt door het kleine aantal studies per onbekend gen, de kleine steekproeven, en de verschillen in de geteste anatomische gebieden en in de methodologie van morfologische metingen.

De voortdurende ontwikkeling van nieuwe en meer complexe genetische en neuroimaging benaderingen heeft het gebied van 'imaging genetics' echter een nieuw tijdperk ingeleid. In mijn promotieproject heb ik verschillende nieuwe statistische benaderingen gebruikt voor het analyseren van de genetica van complexe herseneigenschappen. Dit heeft verschillende mooie resultaten opgeleverd. Ten eerste is er een set van genen, die geassocieerd is met cognitie, gevonden die verschillen in grijze stof in de prefrontale hersenen kan verklaren. Verder is er een nieuwe koppeling gevonden tussen genen die betrokken zijn bij myelinisering en witte stof integriteit en een koppeling tussen genetische risico scores voor schizofrenie met witte stof integriteit. Ten slotte heeft mijn werk ook een verband aangetoond tussen microRNA-137 genen (een bekende genetische variant gekoppeld aan schizofrenie) en de putamen, een hersenstructuur die betrokken is bij verschillende neuropsychiatrische aandoeningen.

Mijn proefschrift laat ook de complexiteit van 'imaging genetics' zien. Het analyseren van grote hoeveelheden data van zowel neuroimaging als genetische informatieve maken het noodzakelijk om te corrigeren voor meerdere testen. Tevens zijn de computationele voorzieningen noodzakelijk voor dit type analyses een grote uitdaging. Het corrigeren voor meerdere testen in neuroimaging analyses vergt dat er een heldere statistische drempel wordt vastgesteld, gegeven het feit dat veel van deze tests niet noodzakelijk onafhankelijk zijn.

In dit project heb ik geprobeerd om de hoeveelheid genetische gegevens samen te voegen door het groeperen van genen in sets met eenzelfde biologische functie. Ook de neuroimaging data heb ik proberen te beperken door alleen die de phenotypes te gebruiken die eerder zijn geassocieerd met een specifieke psychiatrische aandoening. Ik gebruikte echter ook zeer strenge statistische correctie methoden om ervoor te

zorgen dat onze bevindingen niet alleen te wijten waren aan kans. Deze strenge correctie maakte de mogelijkheid om signalen te ontdekken echter aanzienlijk minder.

Toekomstige ‘imaging genetics’ studies zullen gebaat zijn bij de reductie van data op een zinvolle manier en het vergroten van steekproeven. Nieuwe statistische technieken zullen hopelijk ook de kans op het vinden van signalen met klein effect vergroten. Een manier waarop dit kan worden bereikt is door gezamenlijke inspanningen, zoals dit momenteel bijvoorbeeld wordt gedaan door het psychiatrische genetische consortium en het ENIGMA-Consortium. Toekomstige ontwikkelingen in de ‘imaging genetics’ zullen bijdragen aan het ophelderen van de neuronale basis van complexe herseneigenschappen zoals cognitie en ernstige neuropsychiatrische aandoeningen, zoals schizofrenie, bipolaire stoornis en autisme.

About the Author and List of Publications

ABOUT THE AUTHOR

Ivan M. Chavarria Siles is originally from San José, Costa Rica. After completing Medical School at the University of Costa Rica in 2003 he became very interested in genetics while he was being mentored by Dr. Henriette Raventós Vorst.

In 2003 he moved to Madrid, Spain to complete a Master's program in Biochemistry that focused on genetics. In 2005 he obtained his Master's degree, studying the cannabinoid system in an animal model of neuroinflammation.

After completing his Masters degree he moved to San Antonio, Texas to start a NIMH/ Fogarty founded fellowship in psychiatric genetics research, under the Supervision of Dr. Michael Escamilla, and Dr. Henriette Raventós Vorst; his research interest during those years focused in finding genes for Schizophrenia.

In 2010, he moved to the Netherlands to start his PhD training in neurosciences under the supervision of Dr. Danielle Posthuma at the Complex Traits Genetics Department of the VU University in Amsterdam; half way into his PhD, he decided that he wanted to translate some of the knowledge he acquired during his research career into clinical care of patients with psychiatric disorders.

In 2012 he found the place to do this in the Psychiatry Residency Program at Mount Sinai Hospital in New York, where he was able to get clinical training, as well as protected time to continue working on his PhD project in brain imaging genetics. More recently he has extended his research interest by using functional Neuroimaging to study personality disorders.

In 2014 he received the NIMH Outstanding Resident Award, and in 2015 he received the Leon Levy Foundation Neurosciences Fellowship award. He plans to continue his career in academia while caring for patients with various psychiatric disorders.

A. PEER REVIEWED PUBLICATIONS

Ivan Chavarria-Siles, Tonya White, Andrea Goudriaan, Esther Lips, Stefan Ehrlich, Jessica A. Turner, Vince D. Calhoun, Randy L. Gollub, Vincent A. Magnotta, Beng-Choon Ho, August B. Smit, Mark H.G. Verheijen, and Danielle Posthuma. Myelination-related Genes are associated with decreased White Matter Integrity in Schizophrenia. **European Journal of Human Genetics**. 24(3):381-6. **2016**

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B. BOOK CHAPTERS

Ivan Chavarria-Siles, Guillen Fernandez, and Danielle Posthuma. Chapter 8: Brain Imaging and Cognition. **Behavioral Genetics of Cognition Across the Lifespan.** Finkel and Reynolds Editors. Springer **2014**; 235-256. ISBN 978-1-4614-7446-3.

Ivan Chavarria-Siles, Emily Stern, Schahram Akbarian, Pamela Sklar, and Eric J. Nestler. Chapter 4: Translational Neuroscience in Clinical Psychiatry. **Mount Sinai Expert Guides: Psychiatry.** Editors: Simon, New and Goodman. *In press*.**2016.**

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NOTES